BLOOD-STAINS

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St George

BLOOD-STAINS:

THEIR DETECTION, AND THE DETERMINATION OF THEIR SOURCE

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THEIR DETECTION, AND THE DETERMINATION OF THEIR SOURCE.

A Manual for the Medical and Legal Professions.

BY

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LONDON
BAILLIÈRE, TINDALL AND COX
8, HENRIETTA STREET, COVENT GARDEN
1907

TO THE MEMORY OF

MATHIEU-JOSEPH-BONAVENTURE ORFILA,

THE FOUNDER OF MODERN FORENSIC MEDICINE,

AND TO

PAUL EHRLICH,

THE LEADER IN THE EXPLORATION OF THE DOMAIN OF SEROLOGY,

THIS LITTLE WORK IS DEDICATED.

'To Science it matters little whether the birthplace of a discovery be here or elsewhere: our task is to find out whether the report of a new fact is true or false.'—ORFILA.

'If the Law has made you a witness, remain a man of Science: you have no victim to avenge, no guilty or innocent person to ruin or save. You must bear testimony within the limits of Science.'—BROUARDEL.

'He who has had to do with Courts of Law will rightly appreciate this—he will understand how great a moral and legal responsibility the expert takes upon himself when he answers the question as to the presence of blood in the affirmative: on his answer may depend the honour and freedom, even the life, of the accused person.'—MINOVICI.

PREFACE

As there does not exist in any language of which I have knowledge a compendium of the modern tests by which the detection of blood-stains and the determination of their source may be carried out, I have endeavoured to supply this want by putting at the disposal of the Medical Profession, the Bench, and the Bar a full account of these tests. I have at the same time traced the rise of the jurisprudence of blood-stains, for the older tests are valuable in that they show that in the path that leads to truth there are many pitfalls, into which not a few in their eager haste to reach the goal have fallen, and which it is well to mark.

To the reader—especially the non-medical reader—of too many of the text-books of legal medicine that have appeared in the English language, the impression conveyed by their perusal must be that by chemical means alone, without the aid of spectroscope or microscope, it is easy enough to determine whether a given stain is or is not due to blood; and that it is wellnigh impossible, with the aid of all means at our command, to say whether this blood has been shed by man or by another mammal. That the real state of affairs is not quite this I believe that I have shown.

Much that I have written will be new to many of my readers: the precipitin test has not received in text-books written in English that attention which it merits, and the alexine-fixation or complement-deviation test is new, and on its trial. Founded as it is on the sure basis of demonstrable facts of serology, it is bound to be accepted by all courts in the end.

With but one or two exceptions, none of the cases which I have culled from the literature have been mentioned in any British or American text-book of legal medicine, and therefore may be taken to form an addition to our knowledge of the subject, as do those cases which have been privately communicated to me by the observers, whose names are mentioned against them, and to whom and to Their Excellencies the Ministers of Justice in the various countries from which the reports have come, my best thanks are due for the great kindness which they have shown me.

In the bibliography I have only cited those works which have a distinct bearing on the statements made in the text. I have *read* all the works cited, and many more, save those marked with an asterisk, which were not accessible to me in the original. I have had some unpleasant surprises when engaged in verifying the references of others, and desire to spare the reader a like experience. Only those authorities of whom more than one work is cited have been specially noted by number in the text. As the bibliography is set forth in the alphabetical order of the authors' names, I have not taxed the compositor and cumbered the page by making a numerical reference against every author's name, and yet have made the work of reference sufficiently easy, I believe.

It may be that some passages that I have written appear to desecrate the idols of the market-place, whose fault, not mine, it is that their works have not stood the test of experience. I have written what I believe to be true, and it matters little whether the application of my words may seem to entail grave results, for Truth must prevail.

Long ago Domenico Meli wrote of the observers that had gone before him that their writings were 'a formless mass of judgments and opinions, some ludicrous and some absurd, in accordance with the defects of the sciences auxiliary to forensic medicine and the errors and prejudices of the time.' How great an advance has been made since he wrote in one branch of medical jurisprudence I have tried to show in these pages.

To workers in the tropics and in the colonies, where

libraries are few and but poorly filled with works of reference, I hope that what I have written will be of service, as a guide to the performance of the various tests whose value is established, and that thus the interests of Justice may be served.

The sections which deal with the chemical, spectroscopic, and microscopic tests are based on a dissertation which I presented to my Alma Mater, and which was accepted.

In conclusion, I would note that, without the aid afforded me by Dr. Hans Sachs, Member of the Royal Institute for Experimental Therapeutics, and Professor Eugen Albrecht, director of the pathologico-anatomical department of the Senckenberg Institute in Frankfort-on-Main; by Professor G. H. F. Nuttall, F.R.S., of Cambridge; by Dr. E. v. Eisler, of Vienna; by Mr. C. R. Cleveland, C.I.E., Inspector-General of Police in the Central Provinces of India; and by Mr. R. I. Pocock, Superintendent of the Zoological Society's Gardens in London, I could not have carried out my work. It is my pleasing duty to express my thanks to all these gentlemen for the aid which they have so kindly given me.

That in this country the subject of medical jurisprudence has until recently possessed but little interest for members of the medical and legal professions is shown by the fact that the Medico-Legal Society of London is in its infancy. In America the Medico-Legal Society of New York has long since reached maturity, and its Transactions contain much that is of value to the student, and compare favourably with the medico-legal periodicals which have for years appeared in the French, German, and Italian languages.

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September, 1907.

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CHAPTER I

THE SOLUBILITY OF BLOOD-STAINS

EVERY one knows what freshly shed blood looks like, but it is not easy to determine that a stain on cloth, wood, iron, glass, stone, etc., is really due to blood. The colour of the stain may be of any hue from dull red, through reddish-brown, to a dirty grey; for it may have long lain exposed to light and air, or may have been washed out by rain, or by the efforts of the accused person to get rid of the evidence of his guilt. In some cases the stained fabric may be stiffened at the stain, whose surface shows a dull red waxy deposit; in other cases it is only when the fabric is examined by artificial light, or through an eosin film as recommended by Popp, that the stains can be made out. In some cases an extract of the stain is obtained at once by means of distilled water; in others it is only after prolonged treatment with various solvents that an extract can be obtained for examination. In some cases substances other than blood may be present, and mimic blood-stains; in others the stain may look like a rust spot, and yet be a blood-stain.

From the forensic point of view the important constituents of the blood are: (1) The erythrocytes—the red cells—whose form may enable us to distinguish mammalian from other blood, and whose pigment—hæmoglobin—by its chemical

and physical characters enables us to affirm that blood is present or absent; (2) the serum—the watery portion of the blood—which by its chemico-biological reactions, dependent on the albuminous substances which it contains, enables us to fix the source of the blood as to whether it has been shed by a man or other animal; (3) the leucocytes—the white cells—have not yet been found to be of service in solving the questions which occur in forensic medical practice, although certain ideas regarding them have prevailed.

That blood-stains *in themselves* may be of great importance the following cases tend to show:

1. FERRAND.—The body of a man was found in the courtyard of a house, whose inmates declared that he had been drunk and had fallen from a window of the second story. The cause of death was found to be fracture of the skull; but on the landing of the second story was found the man's cap, on the inner side of which there was a small blood-stain, whose position exactly corresponded to the wound on the scalp. The conclusion arrived at was that the man had been knocked on the head and then thrown out of the window.—*Apud* FLORENCE, *Arch. d'Anthrop. Crim.*, 1901, p. 255.

2. FLORENCE and COUTAGNE.—An old woman had been found strangled, no bloodshed having occurred, and suspicion rested on her daughter, who stated that she had not visited her mother for a long time. While she was being questioned by the *juge d'instruction*, he noticed a small blood-stain on one of the ribbons of her head-dress, and when he called her attention to this she became confused, but quickly regained composure, and stated—advancing proofs of her assertion—that at the time of her mother's death she had been menstruating. On the seat of the latrine in the old woman's house there were found blood-stains, pale, and looking as if they had been wiped off. These could not have come from the old woman, and it was concluded that they were due to menstrual blood.—FLORENCE, *loc. cit.*

3. RICHTER.—A man who was accused of having com-

mitted robbery and murder was found to have on the collar, arms, and pockets of his coat, and on his trousers and drawers, blood-stains, which, he stated, were due to his nose having bled. On the left knee of the trousers there was a nearly circular stain, of which, before it had dried, the blood had soaked through the cloth and stained the drawers underneath. In Richter's opinion this stain had undoubtedly been caused by the man's having knelt in a pool of blood.—*Gerichtsärztliche Diagnostik und Technik* (Wien, 1905).

4. Popp.—S. and G. were accused of having committed murder. S. stated that he had only struck the victim once with a 1-kilogramme iron weight, and that it was G. who had murdered him by strangling him with a cord. On the weight was found a blood-stain which bore the impression of S.'s left thumb. On the collar worn by the murdered man was found the impression of G.'s right ring-finger, and on this a pear-shaped blood-drop lay, which had evidently been deposited on the collar after the finger impression had been made. The conclusion arrived at was that S. must have struck more than one blow, and must have had the weight in his hand for some time, while G. must have played a very active part in the murder.—*Zeitschr. f. öffentl. Chemie*, 1904, Heft 19.

5. Popp.—The body of a man was found lying on the floor of one of the rooms of a lodging-house, of which the doors were locked from the inside. Beside the body, which presented a wound on the left side, lay a gun, and the question arose whether the case was one of suicide or murder. On the sill of the window-frame on its outer side were the bloody impressions of the first two phalanges of a thumb, and on the upper side, in a position corresponding to the third phalanx of the middle finger of a hand grasping the wooden frame, were more blood-stains. In the middle of the thumb-impression there was much blood, and on the right upright of a ladder there was a thumb-impression with still more blood, and blood in the position corresponding to the index and middle fingers of a hand grasping the ladder at this spot

Popp concluded from the position and form of the blood-stains in the room that the murderer had fired the fatal shot from a door, and had changed the position of the body after the death of the victim ; and further, that he had a wound, caused by a piece of glass, in the ball of the right thumb. The accused landlord of the murdered man was found to have such a wound, and practically admitted his guilt by hanging himself while the trial was proceeding.—*Loc. cit.*

6. Popp.—A new-born child was killed by being beaten with a boot, on which was found a blood-stain that bore the imprint of five papillary lines of the outer side of the tip of a right thumb. The question was whether the mother of the child or one of the other two women in the house, her mother and sister, had done the deed. By taking a photograph of the impression and enlarging this, Popp was able to determine that the spaces between the lines were 0·413 mm. ; the lines on the mother's thumb were 0·517 mm., and those on the grandmother's and aunt's thumbs 0·555 and 0·455 mm. apart. The conclusion was that the child's mother had killed it.—*Loc. cit.*

But in most cases we shall have to obtain a solution of the elements in the stains, after having carefully recorded the size and position of the stains, eschewing uncertain descriptions such as ' large,' ' broad,' etc., and confining ourselves to giving the exact measurements found—preferably in centimetres. We shall not, however, always obtain an extract of the blood-stain at once : the solubility of the stain depends upon three factors—its age, and the heat and amount of sunlight to which it has been exposed. These factors we shall now consider.

(A) **The Age of the Stain.**—Soon after the medico-legal importance of blood-stains had come to be recognized it was noticed that, while some stains were easy of extraction, others could only be extracted with difficulty, and that the older the stain the greater was the difficulty. So marked, indeed, is this factor of age that Pfaff constructed a scale by which the age of a blood-stain might be determined by its solubility in a 1 : 120 solution of arsenious acid. The scale was this : Fresh

blood dissolves at once; blood one to two days old within fifteen minutes; blood three to eight days old within fifteen to thirty minutes; blood two to four weeks old within one or two hours; blood four to six weeks old within from three to four hours; and blood a year or more old within from four to eight hours. Dragendorff quoted this scale, and Grigorescu was of opinion that it is 'très approximative'; but the experiments carried out by Tamassia (275) with Pfaff's solution and blood-stains of various kinds and various ages—experiments whose result in tabular form I give from Tamassia's article on the subject—ought to set the question of the trustworthiness of Pfaff's scale at rest for ever.

Source of Blood.	Date of Shedding of Blood.	Kind of Cloth, etc., stained.	First Signs of Solution after—		Complete Removal of Stain from Fabric after—
			Days.	Hours.	
Human ...	May 23, 1875	Thin, coarse	2	10	Many days
Human ...	Jan. 8, 1877	Thin, loosely woven	2	0	Ditto
Human ...	April 30, 1881	Fine, but tough	2	12	Ditto
Human ...	June 28, 1881	Coarse, loosely woven	2	0	Ditto
Human ...	June 28, 1881	Sized paper	0	20	After 6th day the paper had become reddish-yellow
Human ...	May, 1883	Coarse	2	12	8 to 10 days
Domestic fowl	Nov. 1, 1871	Fine	1	8	Ditto
Domestic fowl	Jan. 29, 1883	Closely woven	2	nearly	Ditto
Rabbit ...	Feb. 8, 1883	Tattered rag	1	6	Less than 8 to 10 days
Human ...	4 days old	Loosely woven	0	7	Ditto
Human ...	15 days old	Coarse	0	7	Ditto
Human ...	15 days old	Sized paper	0	2	Ditto

(B) **The Heat to which the Stain has been Exposed.**—In 1886 Liman (157) found that he could not obtain a solution of some blood-stains in which he had found erythrocytes. The stains were on a coat which had been ironed after it was stained, and from his experiments he concluded that their exposure to the heat of the iron was the cause of the insolubility of the stains. In 1888 Katayama found that when

bloods that had been dried in porcelain dishes were exposed to a temperature of 80° C., no matter for how long, or to 100° C. for an hour, they were still soluble within twenty-four hours in (1) distilled water; (2) cold saturated solution of borax; (3) ammonia solution; (4) solution of cyanide of potassium; (5) caustic potash solution of specific gravity 1017, diluted with thrice the quantity of water just before use; (6) dilute sulphuric acid 1 part in 20 parts of absolute alcohol; (7) glacial acetic acid. He found that bloods that had been exposed to heat of 120° C. for an hour were soluble within twenty-four hours in Nos. 5 and 7, and within a week in Nos. 3, 4, and 6, while bloods that had been heated to 140° C. for an hour were soluble only in Nos. 5 and 7. In 1892 Hammerl found that blood that has been exposed to heat of 135° to 143° C. loses its power of reacting to the van Deen test, and that when exposed to heat of 140° to 145° C. it loses its power of furnishing Teichmann's crystals. He came to practically the same conclusions as had Katayama regarding the solubility of heated blood, and, like him, found that blood that has been heated to above 160° C. is more soluble than blood that has been heated to temperatures between 110° and 160° C.; he also found that blood-stains on a hard surface—such as glass, iron, or wood—may be exposed to a temperature of 200° C. for hours, and will yet show some erythrocytes, which under ordinary conditions are destroyed by a temperature of 56° C., and will always yield an extract for spectroscopic examination on being treated with glacial acetic acid. And Hofmann, in 1873, had found that heat of 150° C. did not always destroy dried blood-stains.

When the serological tests came to be tried, the question of the solubility of blood-stains naturally became of great importance. In 1901 Ferrai reported, and in 1902 Biondi confirmed the observation, that blood that has been exposed to a temperature of 130° C. for an hour, 140° C. for twenty minutes, 150° C. for ten minutes, or 160° C. for five to ten minutes, yields no extract from which a serum reaction can be obtained. And in 1901 Mirto found that blood that has been exposed to a temperature of 100° to 120° C. for an

hour, 130° to 140° C. for thirty minutes, or 150 to 160° C. for ten to fifteen minutes, is still soluble in physiological salt solution, in the last case only sparingly. He also found that a 10 per cent. solution of the carbonate of sodium or potassium is a good solvent for heated blood. Whether, however, it may be used for the precipitin test we shall see later.

Nuttall found that some of the specimens of blood which he had received from tropical countries were insoluble in physiological salt solution, even when left in it at 37° C. for twenty-four hours. He mentions that Hankin, working at Agra, had written to him as follows: 'There is a practical difficulty in carrying out the test here, in the frequent insolubility of blood-stains, which in this climate may be dried at a temperature of 115° F.,* or even more. Blood-stains usually don't give the spectroscopic test when extracted. If more dried or older, they successively refuse to give the hæmin and, I believe, the guaiacum test.'

We shall see later that it depends on the reagents used as regards the spectroscopic tests; as to the guaiacum test it does not matter, as I shall show.

(c) **The Amount of Sunlight to which the Stain has been Exposed.**—This factor is, of course, of special importance to workers in the tropics. Hammerl exposed a piece of blood-stained linen in a porcelain dish to direct sunlight for seven to eight hours a day, keeping it under a bell-jar in the intervals between the exposures to protect it. After three days the stain was insoluble in distilled water; after six days it was insoluble in ammonia solution, the upper side of the cloth having become bleached to a dirty grey colour by the fifth day. After sixteen days the stain was insoluble in solution of cyanide of potassium. After three weeks the under side of the cloth was bleached to the same degree as the upper side had been by the fifth day; no erythrocytes could be made out, and no Teichmann's crystals could be obtained from the stain, which, however, still yielded an extract for spectroscopic purposes with glacial

* 46·1° C.—by no means uncommon in the hot weather in India.

acetic acid or concentrated sulphuric acid. He found that stains on hard surfaces are but little affected by sunlight, just as they are but little affected by heat. As to the effects of heat, I carried out a number of experiments with various kinds of blood, these being conveniently divided into two series. In the first series the blood-stains were quite fresh, and in the second they were some days old. In both series they were on filter-paper. Briefly the results were as follows:

1. *Fresh Stains.*—(1) Dry heat at 99° C. for six hours: The extract was only sparing after five days; on the eighth day it was in greater amount—with distilled water and with 0·85 per cent. salt solution—the spectroscope revealing the presence of oxyhæmoglobin in the extract. (2) 110° C. for two hours: An extract was obtained within twenty-four hours with distilled water and with salt solution. (3) 120° C. for two hours: A very small amount of the constituents of the stains dissolved out in distilled water and in salt solution. With solution of cyanide of potassium the extract was satisfactory. (4) 130° C. for two hours: Practically the same results as at 120° C., save that the cyanide extract was less in amount. (5) 140° C. for two hours: Very little extract with cyanide of potassium solution. In all cases I was able to obtain crystals of hæmatin chloride from the stains, and in all cases glacial acetic acid and concentrated sulphuric acid yielded a good extract. All stains that had been exposed to a temperature of less than 120° C. gave a snowy froth on the addition of hydrogen peroxide.

2. *Older Stains.*—(1) Eight days old, 99° C. for six hours: The results were the same as with fresh stains similarly treated. (2) Thirteen days old, 110° C. for three hours: A fair amount of extract within twenty-four hours with distilled water, and slightly more with salt solution, the difference being more marked on the third day. (3) Twelve days old, 120° C. for three hours: Very little extract within twenty-four hours with distilled water and with salt solution—on the third day, not more than within twenty-four hours with stains exposed to a temperature of 110° C. (4) Twelve days old, 130° C. for three hours: No extract within forty-eight

hours with distilled water or salt solution. Solution of cyanide of potassium gave a satisfactory extract. (5) Eleven days old, 140° C. for three hours: The extract with cyanide of potassium less than in the former case. (6) Eleven days old, 150° C. for two hours: No extract after eight days with distilled water or salt solution. Extract with cyanide did not yield spectrum of cyanhæmochromogen, perhaps because too dilute. (7) Eleven days old, 200° C. for six hours: An extract was obtained with concentrated sulphuric acid, but very little with glacial acetic acid, and none with phenol.

In the first five cases Teichmann's crystals could be obtained, and all stains exposed to a temperature of less than 120° C. gave a snowy foam on the addition of peroxide of hydrogen.

We see, then, that the heat to which a stain has been exposed, if it be below the temperature of boiling water, has really but little effect on the solubility of the stain if time be given for the solvent chosen to act. And temperatures in the tropics, though high enough, as those who have experienced them can testify, would not be likely to exercise much influence on blood-stains even on cotton fabrics, and scarcely any influence on blood-stains on hard surfaces.

For obtaining an extract of a stain the following method, which has received the approval of countless observers, may be followed: If the stain be on a hard surface, such as glass or metal or wood, it may be scraped off with a bright knife or a clean piece of glass—a piece of a broken slide answers admirably—or even with a piece of boxwood, the particles, as they are removed, being allowed to fall on to a piece of white paper, so that none may escape. This is, of course, of special importance where the material for examination is but small in amount. If the stain be on cloth, a portion of the stained part, *and a portion of the unstained part*, should be removed and snipped into small pieces with a clean pair of sharp scissors (it is of service to use only scissors which have lain in absolute alcohol, and have been held in the flame to light the alcohol which has adhered to them). The snips must be as narrow as possible: for one thing we should

economize our material, and for another we should endeavour to aid extraction of the constituents of the stain or the substances present in the unstained fabric. Snips of 1 to 1·5 mm. are broad enough, and the longer they are, the more easily may they be teased out if required.

Vibert (303) and Filippi have recommended that where the stain is small and on a hard surface it may be extracted by building a wall of wax round it and pouring the solvent into the trough thus formed. Personally I have not yet met with a stain which rendered this manœuvre necessary, and therefore I do not recommend this method of procedure, although it has the great weight of Vibert's authority in its favour.

CHAPTER II

CHEMICAL TESTS FOR BLOOD

I HAVE endeavoured to ascertain when the medico-legal importance of blood-stains first came to be recognized, but without success. The older medico-legists, whose works are cited in the bibliography, treat of most things from miracles to slight wounds, but none make special mention of blood-stains. And, as we see, even so late as 1834 the fourth edition of a popular German textbook contained no special reference to them, although in 1817 Orfila had dealt with the chemistry of the blood in his textbook of medical chemistry, and in 1808 Jacopi is said to have managed by the aid of the microscope to distinguish bovine from human blood.

It is from the discussion which took place at the Académie Royale de Médecine in 1828 that I infer that for a considerable time the French experts had busied themselves with the question of the detection of blood-stains. Raspail, who was an unbeliever (and in the end paid for his scepticism by being tried on a charge of having illegally practised medicine*), had stated that he did not believe that by any means, chemical or microscopical, one could distinguish between blood and many other substances, since with a little white of egg and madder dye he could produce an imitation of a blood-stain which defied detection. Orfila's professional reputation was at stake, and he replied at length (206) to Raspail. He said that he had no hesitation in affirming that a stain was due to blood if its extract reacted as follows: (1) A precipitate is caused by passing chlorine

* He was fined 15 francs. Orfila was one of the informers.

gas into the liquid; (2) no change is produced by the addition of ammonia; (3) a precipitate is formed on the addition of a watery infusion of galls; (4) a white precipitate is formed on the addition of nitric acid; (5) a precipitate is formed on the addition of a considerable quantity of concentrated sulphuric acid; (6) no turbidity is caused by the addition of ferrocyanide of potassium; and (7) on the liquid being heated it becomes, if dilute, opalescent, or shows, if concentrated, the formation of a coagulum. Of these seven tests three have survived to this day, but these are used for reasons other than those which induced Orfila to employ them.

He did not reply to Raspail's taunt about the use of the microscope for reasons which will be apparent when we come to discuss the microscopic evidence in the matter of the detection of blood-stains.

As Orfila was head and shoulders above all the chemists of his time, his belief in the resources of chemistry naturally stimulated many observers to make experiments with a view to discover other confirmatory tests for blood-stains.

Barruel's *Test*.—The first test which we have to consider is that of J. P. Barruel, who in 1829 reported that he had by accident found that when concentrated sulphuric acid is added to a blood-stain or to a quantity of blood, in the proportion of $1\frac{1}{2}$ volumes of the acid to 1 volume of blood, a characteristic odour is evolved, which enables us to assert that blood is present, and, what is more, to tell the species from which the blood has been derived. Raspail promptly reported that this test was not trustworthy, since he had obtained the same odour as that evolved by human blood from a mixture of human sweat and sheep's blood. But Leuret, after carefully testing Barruel's powers of discriminating between various bloods, believed in the merits of the test—or in Barruel's keen sense of smell. And in 1835 Orfila, Barruel, and Chevallier, in a case in which they were consulted, applied the test, and reported that some blood that had been found mixed with earth in a wood did not give off the characteristic odour of blood owing to its having absorbed the odour of

rotten wood and moss. They also stated that, in their opinion, two sets of stains that they had examined had come from different sources, because they evolved different odours, but that the stains were of the same age.

The case was that of the murder of a forest guard named Hochet, of which three brothers named Boileau and a man named Darez were accused. The experts were asked to state—(1) whether the blood found in the wood was human blood, and if so, whether it had been shed by the person whose blood had caused the stains on the clothes of Hochet, J. B. Boileau, and Darez; (2) whether the stains on Boileau's clothes were due to human or hare's blood, and whether they were three weeks or four months old; (3) whether the stains on Darez's clothes were due to human or sheep's blood, and whether they were three or five months old; and, finally (4), whether the stains on a piece of cloth that had been found near the blood-stained place in the wood were due to blood, and, if so, whether they were due to the same blood as that which had stained the clothes of the three men mentioned.

This case appears to have been overlooked by Ch. Robin, who in 1858 stated that he had not been able to find a single forensic case in which this test had been employed. In 1853 J. J. E. Barruel withdrew all claims that had been made for his relation's test, which is, however, mentioned by Dragendorff (1881), not merely as a matter of historic interest.

Persoz's *Test.*—Another of the many tests that did not stand the strain of extended trials was that of Persoz. In 1845 Orfila stated that six years previously Persoz had, according to his own statement, found a sure means of distinguishing blood-stains from other stains on cloth by treating them with hypochlorous acid, which caused the disappearance of all save blood and rust stains. The latter, he said, could be distinguished by their being bleached by tin chloride and hydrochloric acid, while blood-stains were not altered by the addition of these two reagents in succession. Orfila, however, found that if the acid were allowed to act

on the stains for some time, blood or no blood, they were removed by its action.

Dichroism Test.—I cannot say when this test first came into use, but the older observers laid great stress on the fact that an extract of a blood-stain obtained by the addition of caustic alkali is dichroic, appearing red by transmitted, and green by reflected, light. From this fact were evolved the following tests:

(*a*) *The Caustic Soda Test.*—In 1860 Miquel reported that, as Hoppe-Seyler had observed, if a blood-stain be treated with caustic soda it becomes green, to become again red on the addition of acetic acid in excess, and again green on the readdition of caustic soda. Macnamara in 1873 wrote on this test, praising it, as did Casper at first. But in 1863 Liman (158) had found that fruit-stains—*e.g.*, those made by buckthorn syrup and raspberry syrup—give the same reaction, and Casper in a note to this paper, which appeared in his quarterly, stated that he fully agreed with the conclusions expressed by Liman.

(*b*) BOUTIGNY'S *Test.*—In 1844 Boutigny had reported that when the extract of a blood-stain obtained with distilled water is dropped on to a red-hot silver capsule, the drop of liquid becomes greyish-green and opaque, to become again reddish and transparent on the addition of a drop of caustic potash solution, and again greenish and opaque on the addition of a drop of hydrochloric acid. He believed that this was an excellent test for blood, but, so far as I have been able to discover, no one else employed it.

Another test based on physical properties of the blood was CASANTI'S test. In 1848 Casanti reported that when to 2 parts of dried powdered human blood he added 3 parts of phosphoric acid, he obtained a brilliant liver-coloured homogeneous mass, which did not tend to break down on pressure being applied to it; that with menstrual blood the mass was less resistant; and that no homogeneous mass could be obtained with the blood of the horse, mare, ox, calf, mule, pig, roe-deer, or guinea-pig.

ROSE'S *Test.*—In 1853 Rose reported that he had found

a 'sure test' for blood. The suspected material was fused with caustic soda in a narrow glass tube with sealed end, and the fused material was dissolved in distilled water. To this solution were added a mixture of ferrous and ferric oxides, and hydrochloric acid, the result being the formation of Berlin or Prussian blue. WIEHR in the following year proposed that stains on linen or cotton should be fused with potassium carbonate, and a solution of the fused material in distilled water treated with a ferrous and a ferric salt and with sulphuric acid, Prussian blue being obtained if blood were present. This test was modified by CASPER, who boiled the stained material with caustic potash, evaporated the extract thus obtained, and roasted the residue at a red heat, after which he treated it with the iron salts and the sulphuric acid. We have also—

BRYK'S *Test.*—In a long article on blood-crystals he recommended that washed-out blood-stains should be treated thus, to prove the presence of blood: A fragment of the suspected stain is treated with concentrated sulphuric acid, and the preparation examined under the microscope. In a few minutes the fibres will have become pale green, and gradually light brown, or in those places where there is much blood-pigment present, reddish-brown, brick-red, or pink. In two or three hours the whole preparation will be of a dirty brown colour. Stains due to pus, urine, and mucus, which might be mistaken for washed-out blood-stains, might thus, he stated, be differentiated from these, and to distinguish between blood and bile-stains all that was necessary was to treat another fragment of the stained material with caustic potash. And as a matter of history I must mention—

ERPENBECK'S *Test.*—In 1862 Erpenbeck reported that long experience and repeated experiments had led him to conclude that one could always tell whether blood was present in a stain, and also whether it was human blood or not, by heating some of the stained material in a test-tube to near charring-point [140° C.]. Blood so treated, he stated, gives off a characteristic odour, which he loosely described as being somewhat like that of sweat, but not so strong, being more like the

odour of semen; somewhat like the odour evolved when blood is treated with sulphuric acid, and like that evolved when hair, saliva, or flesh is treated with sulphuric acid or heated, but not so sharp. In 1863 Kemper attacked Erpenbeck on the ground that the conclusions expressed in his paper were 'directly opposed to known and admitted facts'; but Kemper referred merely to Erpenbeck's want of respect for Rose's authority, and not to the above statements regarding heated blood.

Van Deen's *Test*.—We now come to a test about which much has been written—van Deen's test. In 1861 van Deen reported that when a solution of blood is treated with tincture of guaiacum and oil of turpentine, a blue colour is obtained, which he considered to be specific. In 1863 Schönbein wrote on the subject, and to his advocacy, and not to that of Day, the vogue which this test has enjoyed is due, I believe. At first the guaiacum and turpentine were mixed together, and then added to the suspected material. Later it was found that it was better to add the guaiacum first and the turpentine afterwards. Then peroxide of hydrogen and oil of eucalyptus were found to act as well as the oil of turpentine, which should be 'aerated for thirty minutes,' or 'exposed to light and air for a long time,' or 'that which by oxidation has bleached the cork of the bottle in which it is contained,' as various observers have recommended. Liebermann states that the blue is due to the oxidation of the guaiacum into guaiaconic acid, which in its turn is oxidized into guaiac-blue, by catalytic action the oxygen of the 'ozonizer' being conveyed to the guaiacum.

As noted, the test was at first taken to be a specific test for blood; but further experiments showed that a number of substances give the reaction. Taylor, who to the end of his life considered that the test is one 'of considerable probative value,' admitted that the colour is caused by the presence of the following substances, which may act alone: (1) manganate or permanganate of potassium; (2) peroxide of manganese; (3) peroxide of lead; (4) chlorine; (5) iodine; (6) bromine; (7) nitrous acid; (8) hypochlorites; (9) persalts of iron;

(10) ferro- and ferrid-cyanide of potassium; (11) gum acacia; (12) gluten; (13) milk, if unboiled; (14) raw potato pulp; (15) pus.

Since Taylor's articles appeared many other substances have been found to have a like action. In 1880 Selmi reported that in 1871 he had found that the test was fallacious, but had not published his observations then, as he had thought that the test would soon be relegated to the limbo of things best forgotten. He had found that with guaiacum tincture and turpentine he obtained the characteristic blue with (16) chloride of lime, (17) chloride of ammonium, and (18) common salt; while the test was vitiated by the presence of dilute hydrochloric acid, phosphoric, oxalic, lactic or acetic acid, and by cyanide of potassium. Vitali confirmed the admissions of Taylor as to the unreliability of the test in the presence of certain substances. Ogston noted that (19) sweat gives the reaction, Spezia that (20) chromium salts act in a similar manner, and Schaer found that (21) quinone vitiates the test by its presence. Further, Dragendorff reported that he had found that with guaiacum and turpentine the blue is produced by (22) rust, confirmed by Siefert and others; (23) salts of copper, confirmed by the researches of Bourquelot and Bougault, and of Breteau; (24) many plant extracts, confirmed by Siefert and by Cevidalli; (25) decoctions of leather; (26) flannel; (27) some kinds of filter-paper, confirmed by Ladendorf and by Breteau. And Tarugi found that (28) the flour of maize, rye, or wheat gives the blue by reason of the oxydases which it contains, and which may be removed, like those of milk and potato, by boiling.

It had long been known that (29) some spring waters vitiate the test, but it was not till Bourquelot and Bougault reported the fact that it became known that even (30) distilled water may have this effect, probably by reason of its containing a trace of copper. Breteau studied this point very carefully, and reported that when sulphate of copper is present in so small a quantity as 1 part in 500,000 of impeccable distilled water, the blue colour appears with

guaiacum alone after some time at room temperature and more rapidly at 40° C., while with guaiacum and turpentine or peroxide of hydrogen the blue appears rapidly at room temperature.

Strange to say, Selmi's statements have not been noticed in any article in the English language that I have read. Accordingly, I made several series of experiments with impeccable distilled water, using guaiacum tincture, prepared according to Taylor's directions, a fragment being taken from the centre of a lump of resin and dissolved in what I believe to have been absolutely impeccable alcohol, although Utz (300) stated that he had found that it makes no difference whether the resin or the wood be used, nor whether these be fresh or old. As 'ozonizer' I used (1) old aerated oil of turpentine; (2) oil of eucalyptus; and (3) 3 per cent. hydrogen peroxide solution. I found that when 1 part of common salt is present in 600 parts of distilled water that is impeccable, the blue is produced within a *reasonable* time. I do not mean that a blue as deep and marked as that produced by the presence of a fair amount of blood is obtained, but I do insist upon the production of a blue coloration of the solution of salt mixed with tincture of guaiacum, *after* the addition of the 'ozonizer.' Now, as we know, common salt is present in sweat and urine, and I do not see how a test which does not exclude the presence of this very common substance, not to speak of a host of other substances, can be held to be worth the trouble of carrying it out.

That a skilful counsel can make a great deal of a small point when chemical tests are in question, the following anecdote, which Dr. (now Sir) Henry Littlejohn used to relate, will show. During the trial of Madeline Smith for the murder of her lover by poisoning him with arsenic, Christison entered into a detailed description of the chemical tests by which he had determined the presence of arsenic. As this arsenic must have been coloured, according to law, by admixture of indigo or soot, an opening was left for Inglis, the advocate for the defence. He casually asked what were the tests for soot, and Christison replied that he could not

remember them. The chemico-legal evidence for the prosecution was much discredited, in the minds of the jury, by Inglis's skilful treatment of this admission.

I do not think that the colour of the extract of a blood-stain, on which Taylor and Glaister lay stress, should be taken to be a weighty argument in favour of the guaiacum test; but the reader will be able to judge for himself when we come to discuss the opinions of experienced observers as to the merits of the test.

To remove some of the faults of the test, of whose value they had a good opinion, various observers have suggested modifications of its technique.

Döbner is reported [I cannot trace the reference] to have suggested that guaiaconic acid be used in place of guaiacum resin. Siefert suggested that the stain should be extracted with acid alcohol, and the extract boiled to destroy any oxydase that may be present, and then rendered alkaline by the addition of caustic potash and filtered, the filtrate being neutralized or rendered slightly acid, and that then a strong solution of common salt be added to it, to render it heavier than the guaiacum tincture. But, as A. Schulz found, this does not do away with the vitiating influence of potassium permanganate, and, as Ziemke found, it does not affect the presence of cupric sulphate; while, as Selmi found, common salt is of itself a vitiating substance.

In 1893 Weber had used the following technique for the detection of blood in fæces: The suspected material is treated with acetic acid, and the extract obtained is shaken up with ether, the ethereal extract being then tested. In 1903 Rossel advocated this method of treating suspected stains, for, as he found, he thus was enabled to exclude many substances—he did not specify these—which have a vitiating influence on the test.

Schaer in 1898 recommended that instead of a 1 per cent. solution, a 5 per cent. solution of guaiacum resin be used, or the solution recommended by Huenefeld, which may be thus prepared: 15 c.c. old oil of turpentine, 5 c.c. chloroform, 1·5 c.c. glacial acetic acid, and 25 c.c. of alcohol, are mixed

together. He also recommended the use of a solution of guaiacum resin (1 part in 100 parts of an aqueous 70 to 75 per cent. solution of chloral hydrate) a solution of which Wood writes in terms of praise.

Spezia praises the modification of the test which was first suggested by Binda, as he found that macroscopically the test is vitiated by oxydases, which may be removed by boiling, and by the following substances, on which boiling has no effect : rust, iodide of potassium, bromide of potassium, common salt, chloride of ammonium, sulphate of iron, sulphate of copper, and chloride of zinc. The presence of these substances, however, is, he states, well shown by Binda's method of carrying out the test microchemically : for, as he says, when the extract of the stain has dried on the slide we can see the crystals clearly and can distinguish them from blood-flakes. Further, blood-flakes react slowly to the test, being for a time coloured blue only at their edges, while their centre remains reddish-yellow, and they may thus be distinguished from the other substances, save cupric and ferric salt crystals ; for these other substances, with the exceptions noted, become blue throughout at once. Binda's technique, however, has these facts against it : it is microchemical, and therefore open to the objections which microchemical methods may have raised against them, and it does not do away with the vitiating power of *all* crystals. What it aims at doing it does not succeed in accomplishing—the obtaining of a colour-reaction yielded by blood, and by blood alone.

Vitali recommended that the suspected stain be treated with caustic potash solution, and the extract thus obtained acidified by the addition of acetic acid, and that, the guaiacum tincture having been added to the extract, the mixture should be allowed to stand for thirty minutes to two hours. If at the end of this time no blue colour has appeared, oil of turpentine or oil of eucalyptus may be added ; and if now we obtain a blue, in his opinion this is proof of the presence of blood. I doubt it. Ladendorf reported that he had found that when oil of eucalyptus is

used in Vitali's modification of the test, if blood be present the oil becomes blue, while if iron salts be present it remains clear. This bluing of the oil does not always occur in the presence of blood, I find. Willcox insists on the reagents being first tested with what is known to be a blood-stain (private communication).

A. Robin adopts the following technique: He dissolves the guaiacum resin in 95 per cent. alcohol, and moistens a piece of filter-paper with the solution. On the suspected stain he places a drop or two of distilled water, and rubs this to and fro over the surface of the stain with a platinum needle, and then conveys a loopful of the extract of the stain thus obtained to the guaiacum-paper. On the drop thus formed he pours oil of turpentine 'that has been aerated for thirty minutes,' and—the result is best described in his own words —'In the presence of the slightest trace of blood, the spot formed by the suspected stain gradually turns blue. By the use of the filter-paper the action is rendered distinct and unmistakable, and as has been shown in this [a murder] case, can be easily demonstrated to a jury more satisfactorily than by the ordinary methods. A number of other substances which oxidize guaiacum were tried with this method, but in no instance could the reaction be mistaken for the characteristic spot resulting from blood. In the case of iron and other oxidizers [not specified in the report—S.] the blue spot appears at once and gradually fades, while in the case of blood the spot turns blue slowly and persists. I am convinced by the result of experiments [not detailed—S.] that the guaiacum test, performed as outlined above, is as delicate and certain as any test for blood.' Now, though Robin's style is everything that it should not be, his meaning is, I think, clear. Firstly, this technique will not do for every case, I believe; and secondly, and of more importance, filter-paper dipped in guaiacum tincture is of no use whatever, as Breteau, who calls its employment 'le résultat d'une pure illusion,' has shown.

Taylor's method of making blood-prints on blotting-paper, and testing these impressions, was enthusiastically praised

by Day; but I hold that this method of applying the van Deen test has nothing in its favour, for reasons which will at once occur to the reader.

From what has been said, it will be clear that the test has many drawbacks; otherwise so many modifications of it would not have been proposed.

We shall now see what observers of great experience have to say as to its merits in forensic practice.

Kornfeld (1884), Legrand du Saulle (1886), and Emmert (1900), do not mention the test at all, so one may safely infer that their opinion of its value was no higher than mine is now, though at one time—mainly, I believe, on the ground of its so frequent employment in clinical medicine—I believed that the test was a valuable one.

Taylor himself wrote: 'If the stain on the material gives no indication of a red colour, or the spectroscopic test—whatever might be the effect of this [the guaiacum test—S.], or other tests—it would be unsafe to affirm that blood is present.' In other words, the red colour of the stain, and the fact that its extract yields the spectra of hæmoglobin and its derivatives, are the only reliable proofs of the presence of blood in Taylor's opinion.

Lefort (1870) wrote: 'If when the reagents are used in succession no colour is produced, this is certain proof that blood is not present.' The test had a sure negative value in his opinion, which was shared by Liman (1863), though afterwards Casper and Liman came to the conclusion that the test was at its best as a negative test, when other circumstances in the case pointed to the probable absence of blood.

And Tourdes (1878) wrote: 'Our conclusions are these: (1) The blue coloration of the stain by the successive addition of the reagents is of much value as a sign of the presence of blood, when one is sure that the colour is not produced before the addition of the antozone. (2) The absence of the colour with guaiacum alone is a means of distinguishing stains due to blood from those due to saliva, mucus, and other substances which produce the blue without

the addition of the antozone. (3) The blue, if immediate, is not always a proof of the presence of blood, for it may be due to ammonia or pus mixed with blood. (4) The reaction is not specific, and, as a positive sign, is subject to certain restrictions, but is of great value. (5) It is a useful means of search for the slightest trace of blood. (6) If the blue colour does not appear, one may conclude that the stain has not been caused by blood. (7) The reaction is of particular value as a negative sign, and is to be recommended because of its delicacy, simplicity, and ease of employment.'

Tidy (1882) wrote: 'Though the guaiacum test is neat and beautiful, it should never be relied upon *by itself alone* as a positive proof of a stain being blood.'

Garibaldi (1882) was of opinion that the test, which he dismissed in a few lines, is of value as a negative test, while Dragendorff (1881), after detailing the various substances other than blood that he had found to give the reaction, wrote that the test 'should not be left untried, for when it yields a negative result, all other tests are superfluous.' This is not quite the case, however.

Luff (1895) wrote: 'The test is only to be regarded as one suggesting the presence of blood, the proof of its presence requiring the hæmin test and spectroscopic examination, since other substances than blood possess the power of yielding a blue colour with guaiacum and peroxide of hydrogen.'

Wood (1894) wrote: 'The test is of value chiefly as a preliminary test, to prove the absence in any given stain of blood-pigment.' Breteau, however, had shown that the blue, though less intense, may be obtained from a solution of blood from which all the hæmoglobin has been extracted; therefore it is presumably not the blood pigment alone which causes the reaction.

Siefert (1898) wrote that the test 'is everything but a positive test.'

Copeman (1890) wrote: 'This test, which by itself is not to be depended on, as it is given with other sub-

stances such as milk, pus, and urine, as well as blood, is nevertheless extremely delicate, and in conjunction with other tests is very valuable, especially as it is so readily applied.'

Mann (1902) wrote: 'This test is the most trustworthy when it yields a negative result, with the limitation that very old stains may not respond to it, though hæmatin readily does so. In its positive phase, the production of a blue colour, the indication is only to be accepted provisionally; the substance tested *may* be blood, but corroboration is required before a decision is given. Other substances, such as gluten, raw potato, milk, bile, and various oxidizing agents, possess the property of striking a blue colour with guaiacum and peroxide of hydrogen; therefore on no account must a positive opinion be expressed from the indications obtained by this test. Even a negative result must not be considered final, as it is an axiom in forensic medicine that every detail demands every possible corroboration. The guaiacum test is therefore only a preliminary test, which is easy of application, and requires but a fragment of material; its use is to pave the way for further inquiry.'

Glaister (1902) is of opinion that 'there is no coloured substance which gives this reaction, and the test is, moreover, an exceedingly delicate one.'

Draper (1905) is of opinion that 'the guaiacum test is a valuable negative test.'

Kockel (1905) holds that 'the guaiacum test is not, either in its original form or in Siefert's modification of it, impeccable and of use for forensic purposes.'

The reader will have already perceived that the earlier writers had a higher opinion of the value of the test than had the later writers, who from the results of further inquiry had derived more knowledge of its fallacies.

As already indicated, my own opinion is that it is well to dispense with a test which has so little value as this test has, in order to save time and trouble and to avoid the possibility of doubt being thrown upon the expert medico-legal evidence, on the ground of one of the tests employed being

inconclusive, a doubt which might obscure the real merits of the other tests performed; for it is not every juryman who can clear his mind of the impressions produced by the brilliant oratory of counsel, and we must remember that when the case is a bad one the oratory to be of any service must be brilliant.

CHAPTER III

CHEMICAL TESTS FOR BLOOD—*Continued*

THE *ZAHN*-GANTTER *Test.*—In 1871 *Z*ahn called attention to the fact that when blood-stained material is brought into contact with hydrogen peroxide a reaction takes places, which is rendered very evident by the evolution of numberless gas-bubbles.

This fact appears to have been lost sight of till Gantter, in 1895, noted that the reaction was of considerable negative value, the absence of the snowy froth being good presumptive evidence of the absence of blood from a stain. In 1905 Palleske, who had not seen Gantter's paper, but was led by the report of an otologist named Richter to perform experiments with the reagent, reported that he had obtained a distinct positive reaction when the solution of blood tested contained only 1 drop of blood in 1,500 c.c. of water. He worked with human blood and with the blood of the ox, pig, domestic fowl, pigeon, duck, frog, blind-worm, earth-worm, fish and flies, and always obtained positive reactions with these, but not with fly-stains. Glaister considers the test, which he calls Gantter's, a good negative test. I have kept Gantter's name for it, although to *Z*ahn belongs the honour of first calling attention to the reaction. As the result of my work with this test, I am able to confirm the statement that many animal and vegetable substances give the positive reaction. I believe that the test is of great negative value, save where the blood-stains have been heated to above 120° C., which is but seldom indeed in practice. Cotton reported in 1904 that he had measured the amount of oxygen evolved

when different bloods were treated with hydrogen peroxide, by adding 1 c.c. of defibrinated blood to 250 c.c. of 12 volume-H_2O_2. The volumes of oxygen evolved were these: for human blood, 600; horse's blood, 300; ox blood, 150; and sheep's blood, 60. No one, so far as I have been able to discover, has tested Cotton's conclusions.

SONNENSCHEIN'S *Test.*—In 1872 Sonnenschein recommended that the solution of the suspected stain should be treated with a saturated solution of tungstate of sodium (acidified by the addition of acetic acid), which produces a voluminous reddish-brown or chocolate-coloured precipitate if blood be present, the iron as well as the albuminous constituents of the blood being thrown down. This precipitate, when heated, forms small masses which are soluble in strong alkali, giving a dichroic solution, from which they may be reprecipitated by the addition to it of an acid. He recommended that these masses should be subjected to Rose's test. Wood recommends that they be tested for Teichmann's crystals.

We have also two recently-devised tests:

SCHAER'S *Test.*—In 1900 Schaer reported that when a solution of a blood-stain is mixed with a 1 to 4 per cent. solution of Barbadoes aloin, and some oil of turpentine is added to the mixture, a red colour soon appears, which in a few minutes becomes cherry-red, and so remains. Other substances give only a pink colour after one to two hours, and are thus to be distinguished from blood. Utz (300) considers that this test, which he found may be performed with hydrogen peroxide, is of considerable confirmatory value. I have tried it with oil of eucalyptus, as well as with oil of turpentine and hydrogen peroxide. The reaction appears to be slower with the oil of eucalyptus. The aloin tincture should always be freshly prepared, as it rapidly undergoes the colour change of itself. I think that the test is a good confirmatory negative test, but further observations as to its merits are required.

The ADLERS' *Test.*—In 1904 O. and R. Adler recommended that the suspected stain be moistened with a con-

centrated solution of leucomalachite green, and then some 3 per cent. solution of hydrogen peroxide be applied to the moistened stain, when, if blood be present, a brilliant green colour will be struck. They state that if no green colour is obtained one may be sure that no blood is present, and that the delicacy of the test is lessened by the presence of uric acid, while the presence of iron salts and nitrates vitiates it.

I believe that this is a good negative test, but further observations are required before it can be admitted into forensic practice. I had made a very dilute solution of the reagent with absolute alcohol, but later Messrs. Grübler and Co. informed me that a solution in acetic acid may be obtained. However, as far as I can see, the alcoholic solution suffices.

Now we come to consider a most important test—

Teichmann's *Test.*—So long ago as 1853 Teichmann reported that when blood is treated with glacial acetic acid in the presence of a minute quantity of common salt, and the preparation is gently heated, peculiar crystals—brownish rhombs—are obtained. These he called crystals of hæmin, but they are now known to be crystals of hæmatin chloride, their formula being $(C_{32}H_{31}ClN_4FeO_3)xC_5H_{12}O$, according to Küster, and their production being due, according to Richter, to the change of hæmoglobin into hæmatin, which then becomes chlorized, and finally crystallizes out. They are of great importance, for they are *a sure proof of the presence of blood* in a suspected stain. Consequently they have occupied the attention of many observers, who have devised various modifications of the technique of their production, some of which I here transcribe from an interesting paper by Lewin and Rosenstein, for the benefit of the curious in these matters (see pp. 30-33).

As the crystals are not easy to obtain in all cases—as, indeed, the various modifications of the test go far to prove—I have thought it well to detail a method which has yielded good results in my hands. On a clean slide is placed a drop of salt solution—the ordinary 0·85 per cent. solution will do as well as any other—and this is evaporated by heating over the Bunsen flame. On the white spot left by the drop are

placed scraped fragments of the stain, or a well-teased small fragment of the stained material, and to the preparation is then conveyed a drop of glacial acetic acid by means of a glass rod. The preparation is then covered with a cover-glass, and some more acid is allowed to run in under the cover, after which the preparation is gently heated until bubbles appear, when it is put aside for a time, being slightly tilted so that the liquid may collect at one part. It is then examined under a magnifying power of × 200 to 300. The following points are of importance: There must be no moisture present, as what is required is glacial acetic acid and not its hydrate; the heat should be gentle, for if the albumin be coagulated we shall obtain a nasty smear instead of a clear preparation; and the longer the acid takes to evaporate the larger will the crystals be if blood be present.

In a private communication Wilcox writes: 'In order to get a positive result with this test, if blood is present, it is my experience that it is most important to take a portion of the dried stain—*e.g.*, a scraping, if on wood or metal, or a piece of the superficial surface of the stained clothing, carefully removed by fine scissors and forceps. The substance removed is placed on a microscope slide, and covered with a cover-slip. Glacial acetic acid is added, and the slide gently heated over a spirit-lamp until bubbles appear under the cover-slip. As the acid evaporates a fresh portion is added, and the slide again carefully heated. This is done usually about three times. On cooling the slide is carefully examined under a $\frac{1}{6}$-inch objective for the characteristic hæmin crystals. If an old stain is dissolved in water, and then the solution evaporated to dryness at about 30° C., and the residue treated as above described, it has been my experience that sometimes no hæmin crystals are obtained even if the stain is blood, owing to the insolubility of the dried blood-pigment. If, however, the stain is removed in the dried condition, and the test applied as I have described, crystals are always obtained if blood is present.'

Husson's method of obtaining the crystals will be described later. It has yielded good results in my hands. It will be

TABLE OF THE VARIOUS PROCESSES RECOM-
PREPARATION OF TEICHMANN'S CRYSTALS,
(*Archiv f. path. Anat.*, 1895,

Authority.	Pure Blood, Fluid or Dried.	Prepared Blood.	Acetic Acid.
TEICHMANN: *Zeitschr. f. rat. Med.*, 1853, 3, p. 375	Dried	—	Much
BÜCHNER and SIMON: *Archiv f. path. Anat.*, 1858, 15, p. 50	Fluid or dried	—	In slight excess
VIRCHOW: *Archiv. f. path. Anat.*, 1857, 12, p. 334	Dried	—	Enough to fill the space under the cover-glass
EYSSAUTIER, *apud* MORACHE: *Ann. d'Hygiène*, 1881, 5, p. 17	Fluid	—	3-4 drops; dilute with four times its volume of water
MORACHE: *loc. cit.*	—	—	Yes
JANERT: 'Die Hämin-krystalle,' *Inaug.-Diss.*, Greifswald, 1875	Dried	—	A few drops
MIALHE, LEFORT, MAYET, and CORNIL: *Repert. de Pharm.*, 10, vii. 73	Dried	—	—
BRÜCKE: *Wiener med. Woch.*, 1857, p. 425	Dried or fluid	—	Yes
AXENFELD: *Ann. di Chim. e di Farmacia*, 1887, 5, p. 98	—	—	—
HOPPE-SEYLER: 'Handbuch der physiol. und path. chem. Analyse,' 1893	Fluid	—	10-20 drops
BIKFALVI: *Cent. Bl. f. d. med. Wissenschaften*, 1886, No. 17	Dried chlorine-free	—	Yes
TEICHMANN: *Zeitschr. f. rat. Med.*, 1856, Bd. viii.	—	Sediment from blood, washed with water. Precipitate thrown down by cupric sulphate, and extracted with sulphuric acid alcohol	Sufficient

MENDED BY DIFFERENT AUTHORS FOR THE GIVEN BY LEWIN AND ROSENSTEIN Bd. cxlii., p. 136 *et seq.*).

Common Salt.		Temperature.	Other Acids.	Other Salts.	Magnification required.
Required.	Quantity in Solution.				
No	—	20°-50° R. (25°-62·5° C.)	Oxalic, tartaric, citric, lactic	—	—
Only when blood deprived of its salts	The smallest particle	In the cold, or 40°-60° C.	—	—	×300
—	About half the quantity of blood	Heat till vapour given off over a flame	—	—	—
—	1 drop of a 1 in 200 solution	Heat, but not up to boiling-point	—	—	—
The salts of the blood sufficient	2-3 drops of 1 in 1,000 solution	Gentle heat, or spontaneous evaporation	—	—	×800-1,200
No	—	Heat till bubbles appear	—	—	—
—	A crystal	Repeated heating up to boiling-point	—	—	×300-400
—	A few drops of a salt solution	Heat in a water-bath	—	—	—
—	—	—	Hydrochloric, malic, sulphuric, benzoic, salicylic, picric	—	—
Only in the case of dried stains	—	One boiling in water-bath	—	—	×300
Only if blood chlorine-free	—	—	Oxalic and tartaric in alcoholic solution	Bromides of sodium, potassium, and ammonium; iodides of sodium and potassium	—
Yes	—	—	—	Chlorides of barium, strontium, potassium, lithium, calcium, ammonium, manganese, tin, iron, and mercury	—

TABLE OF THE VARIOUS PROCESSES RECOMMENDED TEICHMANN'S CRYSTALS, GIVEN BY

Authority.	Pure Blood, Fluid or Dried.	Prepared Blood.	Acetic Acid.
SIMON: *Archiv f. path. Anat.*, 1859, 16, p. 170	—	Preparation of chemically pure hæmatin	Yes
GUNNING and V. GEUNS: *Chem. Cent. Bl.*, 1871, 35	—	An aqueous solution of blood mixed with zinc acetate, and a red precipitate is obtained	Yes
HUENEFELD: 'Die Blutproben vor Gericht,' 1875	—	Extract, with alcohol and ammonia	In nascent state
STRUVE: *Archiv f. path. Anat.*, 1880, 79, p. 524	—	To dilute solutions of blood-pigment are added ammonia, tannic acid, acetic acid till acid reaction; a precipitate of hæmatin tannate is formed	Yes
GWOSDEW: *Sitzungsberd. kais. Akad. d. Wissenschaften*, Wien, 1866, 53, ii.	—	Defibrinated blood precipitated with carbonate of sodium, and the precipitate dried at under 40° C., and extracted with alcohol	Yes
BLONDLOT: *Ann. d'Hygiène*, 1868, 29, p. 130	—	Blood extracted with ammonia-alcohol	A little, and diluted with water
JANERT: *loc. cit.*	—	Defibrinated blood shaken up with salt solution, and left standing for twenty-four hours; the red sediment dried and powdered	In excess
NENCKI and SIEBER: *Archiv f. exper. Path. und Pharm.*, Bd. xviii., p. 404; Bd. xx., p. 325. *Archives des Sciences Biologiques*, St. Pétersb., 1893, ii. 120	—	The blood-corpuscles that have been precipitated are treated with alcohol, and the precipitate is dried in the air at ordinary temperature, rubbed up, and boiled with a large quantity of amylic alcohol and a little strong hydrochloric acid, rapidly filtered, and allowed to cool	—
HOPPE-SEYLER: *loc. cit.*	—	A solution of hæmatin in sulphuric acid spirit of wine, with addition of water	—
HOPPE-SEYLER: *loc. cit.*	—	Oxyhæmoglobin dissolved in glacial acetic acid	—

BY DIFFERENT AUTHORS FOR THE PREPARATION OF LEWIN AND ROSENSTEIN—*Continued.*

Common Salt.		Temperature.	Other Acids.	Other Salts.	Magnification required.
Required.	Quantity in Solution.				
Yes	—	—	—	—	—
—	—	—	—	—	—
Not absolutely necessary, but advantageous	—	Heat, but not to boiling-point	Formic	—	—
No	—	—	—	Chloride of ammonium	—
Yes	A little	—	—	Chloride of calcium	—
—	—	Heat, but not up to coagulation-point for albumin	—	—	—
—	Not too much	40°-50° C.	—	—	—
—	—	—	—	—	—
—	A little dry	—	—	—	×300
—	A trace of salt	—	—	—	—

noted that Willcox adds no salt, thus following Teichmann's first technique and Janert and Struve's dicta.

Wachholz in 1901 stated that he had found that all concentrated mineral and organic acids may be used for the test, and recommended that a 1 : 10,000 solution of concentrated sulphuric, lactic, or glacial acetic acid in 90 to 95 per cent. alcohol be employed, as this boils easily, and therefore there is less danger of overheating the preparation than by the ordinary method, in which pure acid is used.

As we have seen, Hammerl found that blood that has been exposed to a temperature of 140° to 145° C. will not yield the crystals, and that they cannot be obtained from a blood-stain on cloth that has been long exposed to sunlight. That blood which has been heated to a temperature below 140° C. will yield the crystals I have found by repeated experiment.

Hofmann (116) believed that when blood-stains have been washed with strongly alkaline soap, they become so modified that they do not yield the crystals; and Zanelli found that when blood-stained clothes are washed in the usual way with (1) soap, or with (2) the washing mixture in common use in Italy, which contains a large proportion of potassium carbonate, or with (3) chloride of lime, crystals of hæmatin chloride cannot be obtained from the stains.

Lewin and Rosenstein, on the other hand, concluded that it does not matter whether blood be mixed with a hot solution of soap or boiled with lye—crystals may still be obtained from it.

Blondlot reported that absolute alcohol has an inhibitory effect.

Siefert smeared a piece of rusty iron with blood and left it exposed to the weather on the sill of a window of his laboratory. After five days no crystals could be obtained from the blood on the upper side of the iron; and after fifteen days, from the blood on the lower side, which had been in contact with the stone sill, and thus protected, he could obtain crystals only once out of five experiments. Dragendorff thought that the presence of rust had no influence on the test; but Siefert's experiment, and my own confirmation

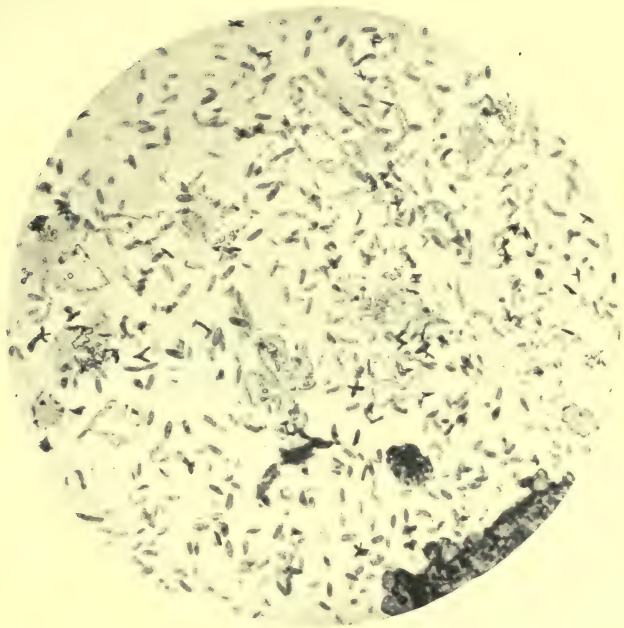

FIG. 1.

CRYSTALS OF HÆMATIN CHLORIDE, FROM A FORENSIC CASE.

Prepared by Dr. Hans Sachs. × 250. The hemp-seed crystals are numerous, and there are several 'crosses' present.

To face p. 34.

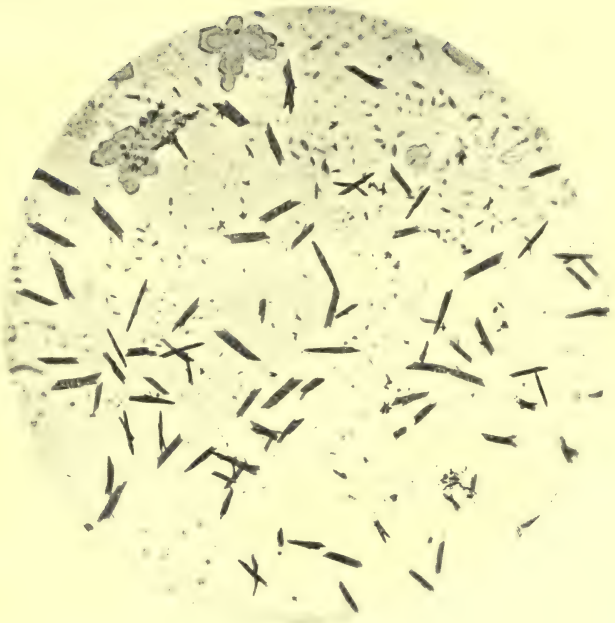

FIG. 2.

CRYSTALS OF HÆMATIN CHLORIDE, FROM A FORENSIC CASE.

Prepared by Dr. Hans Sachs. × 250. Rhombic plates are here in evidence. The ill-defined crystals in the left upper quadrant are those of the sodium salt.

To face p. 34.

of it, show that rust *plus* exposure has a distinct inhibitory effect. Tamassia found that iron and an organic acid had a similar effect within eight to ten days.

Richter found that he could obtain the crystals from blood-stains five months old on (1) linen, (2) a bright knife, and (3) a rusty knife, which had been lying in the laboratory exposed to light, but not exposed to the influence of the weather. He remarked that in many cases where he failed to obtain the crystals at once, by leaving the solution to stand for twelve to twenty-four hours he managed to obtain them.

Lewin and Rosenstein found that no crystals could be obtained from blood that (1) had been mixed with dilute hydrochloric or nitric acid, and dried at 40° C.; or (2) kept in contact with iodic acid; or (3) kept for five to nine days in contact with an excess of chlorate of potassium.

The usual appearance of crystals is that shown in the first microphotogram; in very good preparations large crystals, such as those shown in the second microphotogram, may be obtained, the longer the process of evaporation the larger being the crystals. These illustrations are from a specimen, for which I have to thank Dr. Hans Sachs, who obtained it in a forensic case in which he was consulted.

Virchow suggested that indigo crystals might be mistaken for those of hæmatin chloride; and Vibert (303) reported that Descoust had shown him some indigo crystals, which he had obtained by washing a piece of violet-blue dyed flannel, and which were exactly like crystals of hæmatin chloride, which, as Vibert points out, are insoluble in water, alcohol, and dilute hydrochloric acid, but soluble in boiling acetic or hydrochloric acid, and when incinerated, leave an ash which gives a red colour when treated with a drop of hydrochloric acid and a drop of solution of sulphocyanide of potassium.

In Japan and India, where garments dyed with indigo are commonly worn, the distinction is of importance. As Glaister has suggested, in case of doubt a drop of solution of peroxide of hydrogen may be added to the crystals, which, if of hæmatin, will cause the characteristic foam-formation.

Liebreich, in 1872,* suggested that crystals of murexide might be mistaken for those of hæmatin chloride; but, as Vibert truly says, murexide is not manufactured by the addition of salt and glacial acetic acid to a substance. In all cases indigo and murexide may be excluded by the spectroscopic test.

The following tests are as yet on their trial:

The UTZ-MEYER test was first suggested by Meyer, and brought into forensic practice by Utz (301). It is performed by dropping a fragment of the stain into a solution of phenolphthalin (produced by boiling phenolphthalein with zinc dust in alkaline solution), which, if it be alkaline, is quite colourless, and to which a few drops of a weak solution of hydrogen peroxide have been added. If blood be present, its oxydase will cause the test liquid to become pink. The solution must always be freshly prepared.

RIEGLER'S *Test.*—This is performed by adding to a fragment of the suspected stain some of a solution prepared by dissolving 5 grammes hydrazin sulphate in 100 c.c. of a 10 per cent. solution of caustic soda, 100 c.c. of 96 per cent. alcohol being then added to the solution. If blood be present the fragment of the stain becomes pink.

Palleske believes that when this reaction is obtained we have positive proof of the presence of blood; but Popp employs this and the Utz-Meyer test merely as guides, and not as proofs.

We may now consider the three tests of Orfila's seven which have survived, and are still used in forensic practice.

1. **The Addition of Ammonia to the Stain-Extract.**—This excludes vegetable colouring matters, which have their reddish hue changed to green, crimson, or bluish-black, while blood-pigment is unchanged by the addition of ammonia.

2. **The Heating of the Stain-Extract.**—This gives us proof of the absence or presence of albumin in the extract. Poore wrote that it is *the* test for blood, but few will be inclined to follow him so far.

* At a discussion in the *Berliner med. Gesellschaft*, 23, x. 72.

3. **The Addition of Nitric Acid to the Stain-Extract.**—This serves to exclude (red or reddish-yellow or brown) aniline dyes, which at once become yellow on the addition of the acid, while blood-pigment undergoes no such change of colour.

As to Raspail's mimic blood-stain, its colouring matter was madder, a dye of which the colouring principle is alizarin, which, as Berzelius discovered, becomes violet on the addition of ammonia and yellow on the addition of a mineral acid.

We have further to consider the distinction between blood-stains on the blade of a knife or other weapon and stains which might be mistaken, and have indeed been mistaken, for them.

Rust-Stains.—These are insoluble in water, do not scale off when the other side of the blade is heated, and when treated with a drop of hydrochloric acid are removed, leaving the part clear. Scrapings from the blade, if of rust, will not be found to be stained when treated with Marx' solution, and examined under the microscope.

Fruit-Stains.—These become greenish on being treated with ammonia, and the stains formed by the fruit acids on iron are soft and deliquescent, not hard and brittle as are blood-stains. The blade may be washed with a small quantity of distilled water, and to the solution of the stain thus obtained a drop of nitric acid may be added to oxidize the iron into a ferric compound, and then a drop or two of freshly-prepared solution of sulphocyanide of potassium will give the rosy-red sulphocyanide of iron (Mann).

In any case, whatever be the results of the chemical tests employed, the spectroscope must be used to confirm them, as a matter of routine.

CHAPTER IV

THE SPECTROSCOPE IN THE DETECTION OF BLOOD-STAINS

THE spectra, of which representations are given on the plate, have been drawn by me on Engelmann's coloured plates as faithfully as I could from Formánek's diagrams, controlled by observations with a large Bunsen's spectroscope, etc. The spectra are drawn to wave-length scale, and look clear enough ; but if the reader expects with an ordinary pocket spectroscope to see anything like so clear a picture when examining a solution, he will be very dissatisfied with his spectroscope, or with the plates : for it is only when one has an extensive dispersion of light, such as is obtained with a large fixed spectroscope, that such a picture can be seen. Yet by a little practice the reader will be enabled to read his spectrum aright, and will be pleased to find the various spectra of the modifications of hæmoglobin which he has produced, and to distinguish these one from the other with certainty—the more easily if he uses a pocket spectroscope with wave-length scale, such as is supplied at a moderate cost (£4) by the firm of Carl Zeiss of Jena.

From the work of numberless observers, who carried out countless experiments, it has been established beyond doubt that the spectra of blood and its modifications are constant and characteristic. We are thus enabled to accumulate a mass of proof which not even the most zealous advocate for the defence will dare to question, if he values his reputation.

It will be of service first to study the spectra which may

be obtained from blood itself, so that we may be able to appreciate the various means which have been devised for the examination of suspected stains. The description of the spectra I take from Formánek, whose work is the most recent, and certainly the best that I have read on the subject.

Oxyhæmoglobin.—If we examine a solution of defibrinated blood that has been diluted with water, we shall see a spectrum in which there are two dark absorption-bands between Fraunhofer's lines D and E in the yellow-green, the darker and more clearly-defined band lying at λ 57·8, the lighter and less clearly-defined at λ 54·1. On the right of the spectrum the light has been absorbed from F to the right.

Met-hæmoglobin.—If the blood-solution be left exposed to light and air for some days and then examined, we shall see, in addition to these two absorption-bands, which have become fainter, two others. One of these lies to their left, in the red-orange between C and D at λ 63·4, the other lying to their right, being faintly defined in the green between E and F at λ 50, while the absorption of light on the right of the spectrum has become less extensive. This spectrum is that of a mixture of oxy- and met-hæmoglobin.

Hæmoglobin.—If to our original blood-solution we add some *old* ammonium sulphide or some of Stokes' solution (1 part of ferric sulphate and 1 part of citric or tartaric acid in 10 parts of water, 6 parts of ammonia solution being added just before use), we shall obtain a spectrum in which there is a diffuse broad absorption-band in the yellow, fading towards the left, near D at λ 55·4, the light on the right of the spectrum being absorbed from F onwards. We have also a faint band in the red-orange between C and D at λ 61·9, which is due to the presence of sulph-hæmoglobin. Note that the ammonium sulphide should be old, and contain no free ammonia, else we shall obtain the spectrum of hæmochromogen (*q.v.*).

Hæmatin, Acid.—If to the original blood-solution we add some dilute mineral acid, or tartaric, oxalic, or acetic acid, we shall have the oxyhæmoglobin split up into albumin and

acid hæmatin, and if the solution be concentrated, or we have a deep column of fluid before us, we shall obtain the spectrum of acid hæmatin. This presents two ill-defined absorption-bands near E at λ 55·4 and λ 51·7, which lie at λ 56·5 and λ 52·6, after the solution has stood for twelve hours. There will also be seen a faint band in the red at C at λ 65·4, and this will, after twelve hours, have moved to λ 66·5. The absorption of light on the right will involve the blue. The third band was first described by Stokes, whose name it sometimes bears.

Hæmatin, Alkaline.—If the blood-solution be treated with some caustic potash or caustic soda, we shall obtain (1) *alkaline hæmatin in aqueous solution*, whose spectrum, if the solution be concentrated, will present two ill-defined absorption-bands between D and E at λ 52·8 and λ 54·6, with absorption of light on the right up to the green. If we heat this solution to near boiling-point and dilute it with water, after it has cooled we shall find a single faint band near D at λ 58, fading to the right. If the dilution be carried still further this band disappears. If, instead of water, ethylic alcohol be added to the heated solution we shall have (2) *alkaline hæmatin in alcoholic solution*, whose spectrum presents a single band near D lying between λ 60 and λ 59·7 and fading to the right, while the absorption of light on the right has become more extensive, very little of the green being visible.

The reader will do well to remember that, although many textbooks do not mention the fact, *the hæmatin spectra are the reverse of delicate*. To be seen they require a concentrated solution or a deep column of fluid. Having treated the blood-solution with acid or alkali as above detailed, whether we have obtained the hæmatin spectra or not, we may now proceed to obtain that of **Hæmochromogen,** by adding some sulphide of ammonium to the fluid. We shall then see two absorption-bands, one very well defined at λ 55·9, and the other narrower and fading to the right at λ 52·9 in the green between D and E. The absorption of light on the right will extend from the blue onwards. After the preparation has

stood for twelve hours, the bands will be found lying more to the right, at λ 55·4 and λ 52·5 respectively. This is one of the most delicate spectra of blood that we have—in de Domenicis' opinion it is the most delicate—and it should be always looked for.

Hæmatoporphyrin.—If to some blood we add concentrated sulphuric acid, and gently warm the mixture—using as small a quantity of blood as possible—we shall obtain 'iron-free hæmatin,' as its discoverer, Mulder, called it. This is now known by the name of hæmatoporphyrin, and yields two different spectra, according as it is in alkaline or acid solution.

If the mixture of blood and acid be carefully diluted with a little alcohol and water, we shall obtain the spectrum of (1) *acid hæmatoporphyrin*, which presents two absorption-bands, whose exact site depends on the age of the blood, the time for which the acid has been allowed to act on it, and the degree of heat applied to the mixture. They lie, the narrower and fainter between λ 60·4 and λ 59·9 near D, the broader and better defined to the right of this, between λ 55·8 and λ 55·3, the light on the right being absorbed from the blue onwards.

If to our solution we now add, *with great care*, some caustic potash in excess, we shall with luck obtain (2) *alkaline hæmatoporphyrin*, whose spectrum presents four absorption-bands, of which three are generally visible. The darkest band lies between E and F in the green at between λ 51·1 and λ 50·5, the others lying to the left of this, at λ 54·4 to λ 53·8, λ 57·7 to λ 57, and λ 62·6 to λ 62 respectively, the absorption of light on the right leaving a little of the blue visible.

At one time it was thought that, because the spectra obtained from the wing feathers of the Cape lory, alkanet, cineraria flowers, and from cochineal or madder in alum solution, resemble those of oxyhæmoglobin, the proof afforded by the spectroscope of the presence of blood might be questioned. We now know that when a solution gives with the reagents noted the spectra described it contains blood, and

that no solution which does not contain blood can possibly give these.

As a matter of history, I would note that the first case in the English courts in which the evidence afforded by the spectroscope was brought forward was the trial of Franz Müller for the murder of Briggs in July 1864, not so very long after the appearance of Hoppe-Seyler's work.

As the spectrum of *hæmochromogen* is so very delicate, it has naturally been the endeavour of many observers to devise an easy method for its production when dealing with suspected stains. Donogány suggested the use of pyridin and ammonium sulphide, which reagents de Domenicis (56, 57) praises as being of service with all bloods, fluid or dry, soluble or insoluble, reporting that with their aid he was able to obtain the spectrum from one fibre of a piece of blood-stained cloth. He recommends that the stain scrapings or fragment be treated with 1 drop of pyridin and 1 drop of ammonium sulphide, and the preparation examined with the microspectroscope; or that to 2 c.c. of the extract of the stain there be added 5 drops of each reagent, and the mixture then spectroscoped. He states that even where he failed to obtain the spectrum of acid hæmatoporphyrin, he obtained that of hæmochromogen by these means. Thomas reports that he can find no compensating advantages in this method of dealing with the stain to make up for the abominable odour of pyridin. But Lecha-Marzo employs Donogány's reagents after evaporating the extract of the stain which has been obtained with a 20 per cent. solution of caustic soda, and treating the residue with an alcoholic solution of iodine (potassium iodide 0·5 gramme, iodine 2·5 grammes, 96 per cent. alcohol 25 c.c.). He states that if blood be present, we shall then find doubly-refractile dark orange-red crystals of iodine-hæmatin and hæmochromogen, which may then be examined with the microspectroscope. Corin used boiling pyridin alone for the obtaining of hæmochromogen with success. Dvornitschenko recommends that a fragment of the stain be treated with some 50 per cent. solution of caustic

potash, and the preparation then heated till its colour has changed from brown to bright red, when there will be found large masses of regular outline, somewhat like erythrocytes, but differing from these in size and colour. The largest of these is chosen for examination with the microspectroscope. Florence recommends a similar method of dealing with the stain, the only difference being the time taken in preparation. A. Schulz recommends that the stain be extracted with 10 per cent. solution of caustic potash, and the extract examined for the spectrum of alkaline hæmatin, and then treated with ammonium sulphide, and examined for the spectrum of hæmochromogen. He also recommends as a solvent a solution of cyanide of potassium, which will yield with sulphide of ammonium the spectrum of cyanhæmochromogen, which closely resembles that of oxyhæmoglobin, but is, of course, easily to be distinguished from it, since it is produced by the action of a *reducing* substance.

Szigeti reported that he had found that pure anhydrous phenol, with or without the addition of absolute alcohol, is an excellent solvent for stains, even if they be twenty years old, or have been exposed to a temperature of 200° C. He heated the stain fragment with the solvent to boiling-point, filtered the extract through filter-paper, and examined the filtrate for the spectrum of acid hæmatin. Then he added some 30 per cent. solution of caustic potash, or, if alcohol phenol had been used, some sulphide of ammonium, to the filtrate, and examined it for the spectrum of hæmochromogen, the whole process being carried out microspectroscopically if only a minute fragment of the stain were available.

Willcox, in a private communication, writes: 'A portion of the stain is placed in a test-tube, and about 1 or 2 c.c. of distilled water added, and the tube kept at 35° C. for about half an hour. The liquid is then cleared. This can be done by filtration, or, when only a small quantity of liquid is available, it is more convenient to centrifugalize the liquid and remove the clear supernatant solution. The clear liquid is examined by the microspectroscope for the spectrum of

oxyhæmoglobin, or met-hæmoglobin, or reduced hæmoglobin, the characteristic absorption-bands being present as the case may be. In very old stains sometimes the spectrum of hæmatin, acid or alkaline, may be seen. Afterwards the liquid is treated with about 4 drops of a 25 per cent. solution of caustic soda, and 2 or 3 drops of ammonium sulphide solution, and the mixture warmed to about 60° C. for two or three minutes. The liquid is cleared, if necessary, by centrifugalizing, and the clear solution is then examined by the microspectroscope, when the characteristic absorption-bands of hæmochromogen *will* be visible if blood is present. It has been my experience that often with very small blood-stains the spectrum of hæmoglobin, met-hæmoglobin, or hæmatin in the preliminary examination is very faint, but that with these weak solutions, on conversion to hæmochromogen, the characteristic absorption-bands of this body will appear distinctly, the first absorption-band being the more marked.'

Richter macerates the stain in distilled water for one to two hours, using only 0·5 c.c. of water, the tube being 5 cm. long, by 0·7 cm. in diameter. If he obtains a yellowish solution, he spectroscopes this for oxyhæmoglobin. If the two characteristic bands be seen, he proceeds to try to obtain hæmoglobin; if they are not seen, he adds caustic soda or potash in solid form, so as to avoid dilution, and gently heats and spectroscopes for alkaline hæmatin, and then adds ammonium sulphide, and spectroscopes for hæmochromogen, whose line near D is, he states, narrower and darker than that near E. Or he adds solid cyanide of potassium, and spectroscopes for the single band of cyanhæmoglobin, cyanmet-hæmoglobin, or cyanhæmatin, and, after adding ammonium sulphide, for the two bands of equal intensity, lying close together, of cyanhæmochromogen.

Ipsen devised the following method of dealing with refractory stains: Into the Erlenmeyer flask which contained the suspected material he poured some 98 per cent. absolute alcohol, and added to this some cupric sulphate that had been roasted in a sand-bath. The contents of the flask were left to stand for several days, until the supernatant alcohol

had become distinctly coloured. The coloured liquid was then placed in long tubes, and these were so arranged that the full depth of their contents could be spectroscoped for acid hæmatin. To the extract he then added an alcoholic solution of caustic potash and spectroscoped the mixture for alkaline hæmatin in alcoholic solution, and then, on the addition of sulphide of ammonium, for hæmochromogen. Or, instead of the cupric sulphate, he used pure acetate of potassium (10 grammes to 100 c.c. of 98 per cent. alcohol), and spectroscoped the coloured supernatant fluid for alkaline hæmatin in alcoholic solution. He stated that this method of treatment gave good results even with charred blood.

Puppe recommended that old stains be macerated in a mixture of equal parts of formalin and absolute alcohol, which he had found extracted all stains save those that had been exposed to 180° C. or more. He used a 40 per cent. solution of formalin.

Richardson recommended glycerin as a solvent for microspectroscopical work; but Tidy found that this does not answer in the case of old stains.

Cevidalli extracts the stain with boiling ammonia solution, and to this extract, which must be as concentrated as possible, he adds a drop of piperidin ($C_5H_{11}N$), and obtains crystals of hæmochromogen, whose forms may be rhombic, rectangular, acicular, or barrel-shaped. These he examines with the microspectroscope, and obtains the spectrum of hæmochromogen, or at least its first absorption-band.

Though Mulder had discovered *hæmatoporphyrin* in 1844, and Struve in 1880 had noted that its spectrum would be of use in forensic medicine, it was not till 1892 that it began to be generally used in forensic practice, after Kratter had published the results of his experiments. He recommended that to a fragment of the stain be added a drop of concentrated sulphuric acid, and that the mixture be squeezed between two microscope-slides, the thickest part of the layer between them being then spectroscoped. Naturally, if carbonized fragments of stained material be present, they interfere with

the examination, which Ipsen had suggested might be rendered easier by washing the preparation and adding more acid. This manœuvre was found to be of little use, however, so Ziemke devised the following method of removing the carbonized fragments : After the acid has been allowed to act on the stain for twenty-four hours, the extract is filtered through glass-wool, and the filtrate neutralized with ammonia, which produces a copious brown flocculent precipitate, which soon settles at the bottom of the tube. This precipitate is then washed with distilled water, filtered, and dried in the air. The dried residue is rubbed up in a mortar with equal parts of absolute alcohol and strong solution of ammonia, and the mixture is then filtered, and the filtrate examined for the spectrum of alkaline hæmatoporphyrin.

Takayama allows the acid to act on the stained material for five to seven days, using as little acid as possible, so that he may eventually have a concentrated solution for examination. He heats the extract in the spirit-flame for ten to fifteen seconds, or places the tube in boiling water for three to five minutes, according to the size of the piece of stained material, shaking the contents of the tube constantly during the process of heating; then he adds twice the extract's volume of water, and filters the dilute extract through glass-wool, thus removing any indigo that may be present. The filtrate is then spectroscoped. If it be dilute, only the right-hand band of the spectrum of acid hæmatoporphyrin will be visible, in which case a deep column of the fluid must be used for examination. He notes that when a concentrated solution of acid hæmatoporphyrin is heated, it becomes of a dirty green colour, and gives a third band in the red, more to the right than the third band of acid hæmatin.

Giese recommends that in order to remove all interference which the presence of certain dyes may cause, the spectroscopic examination of a portion of the *unstained* fabric should also be carried out. He believes that Takayama's method of treating the stained material is the best.

The following cases will show the value of spectroscopic examination :

7. IPSEN.—A bundle of woman's clothes and bedclothes was fished out of the River Inn, and, on examination of its contents, some stains were found, which were suspected to be due to blood. Owing to the presence of moulds, it was impossible to dry the stained material thoroughly, and chemical and microscopical examination of the stains yielded negative results; but by means of his method of treatment Ipsen was enabled to demonstrate the presence of blood in the stains by treating the stained material for *one* day.—*Viertelj. f. ger. Medicin*, 1898, 3te. F. 15, p. 111.

8. IPSEN.—A workman had been murdered, and one of his companions was suspected of having killed him, but denied his guilt. Some stains were found on this man's trousers, which gave negative results with chemical tests and under the microscope, but were found on the *eighth* day to be due to blood on being treated by Ipsen's method and spectroscoped. The man confessed and was sentenced.—*Loc. cit.*

9. IPSEN.—An old rusty pistol, on which were suspected to be blood-stains, gave negative information when tested chemically and examined microscopically; but the presence of blood was demonstrated by Ipsen's method on the *fifth* day.—*Loc. cit.*

10. HERAPATH.—In the case of Reg. *v.* Robert Coe, tried at the Swansea Assizes, 1866, Herapath demonstrated the presence of blood in a stain on the haft of an axe on the part exposed by removal of the blade, although he had less than $\frac{1}{1000}$ grain of blood to work with.—*Brit. Med. Journ.*, 1868, i., pp. 189, 217.

CHAPTER V

THE MICROSCOPE IN THE DETECTION OF BLOOD-STAINS

As already mentioned, Jacopi is reported to have been able in 1808 to determine that some stains which were suspected to be due to human blood were really due to bovine blood; but Meckel, who noted this fact, paid no attention to it, as he considered that the microscope was not needed for medico-legal work. Orfila admitted that, 'as a rule,' mammalian erythrocytes may be distinguished from the erythrocytes of birds, reptiles, and fishes, but stated that in practice he had met with insuperable difficulties in the microscopic examination of blood, being sometimes unable to distinguish the erythrocytes of man from those of a pigeon, or, indeed, to tell that it was really blood that he had before him. As Mandl pointed out, the difficulty of finding a suitable medium for the microscopical preparation was the cause of this want of confidence in the results obtainable by microscopic examination of blood. A number of fluids have been recommended for the treatment of suspected stains, and of these I give here those which appear to have obtained most favour. To name all that have been recommended would be tedious and unprofitable:

1. Pacini's *Fluid:* Common salt 4 grammes, glycerin 26 grammes, mercuric chloride 2 grammes, water 226 c.c.; the fluid to be diluted with two or three times its volume of water before use. This fluid is recommended by Richter.

2. Hofmann's *Modification of Pacini's Fluid:* Glycerin 101 c.c., common salt 2 grammes, mercuric chloride 1 gramme, distilled water 300 c.c.

3. Roussin's *Fluid:* Concentrated sulphuric acid 1 and glycerin 3 volumes, made up to specific gravity 1028 at 15° C. with distilled water.

4. Hayem's *Fluid:* Sodium sulphate 5 grammes, common salt 1 gramme, mercuric chloride 0·5 gramme, distilled water 100 c.c.

5. Dragendorff's *Fluid:* Sodium sulphate 5 grammes, common salt 1 gramme, distilled water 94 c.c.

6. Vibert's *Fluid:* Common salt 2 grammes, mercuric chloride 0·5 gramme, distilled water 100 c.c. Highly praised by Masson.

7. Grigorjew's *Fluid:* Caustic potash 12 grammes, sodium-potassium tartrate 40 grammes, distilled water 100 c.c.

8. Rezzonico's *Fluid:* Saturated solution of tannic acid 1, and glycerin 3 volumes.

9. Puppe's *Fluid:* Caustic potash solution 15 per cent., and 40 per cent. formalin solution, equal volumes.

10. Coraini's *Fluid:* Oxalic acid 10 per cent. solution.

11. Donders' *Fluid:* Caustic potash 32 per cent. solution. Recommended by Virchow, Kölliker, and others, including Malinin.

12. Bizzozero's *Fluid:* Caustic potash 26 per cent. solution.

13. Malassez and Potain's *Sérum artificiel:* Equal parts of 1020 specific gravity solutions of gum, sodium sulphate, and common salt.

14. Lesser's *Fluid:* Tartaric acid 15 per cent. solution.

15. Marx' *Fluid:* Quinine chloride 1 per mille solution in distilled water and 33 per cent. solution of caustic potash, equal volumes, tinted with eosin, which gives a hæmatoxylin colour to the solution, but stains the preparation of the characteristic eosin hue.

So unsatisfactory were the various manœuvres which had been suggested in his day, that in 1848 C. Schmidt recommended that sections of a fragment of the stain should be examined without any fluid being added to them. He said that, in the case of mammalian stains, by micrometry one

could refer the erythrocytes seen in such a preparation to their source. If the reader tries this plan—and he must remember that Schmidt had no microtome at his disposal—he will be convinced that Schmidt either was guilty of exaggeration or was the most perfect microscopist of his own or any other time. Pelikan, whose article I have not read, is reported to have written that to doubt the merits of Schmidt's method was to be guilty of impertinence!

Malinin, for whose article in his quarterly Virchow apologized, stated that he had earned the gratitude of humanity by pointing out that a solution of caustic potash of 32 per cent. strength is a good fluid for preparing blood for microscopical examination, although he admitted that Donders had mentioned the merits of caustic potash.

For the treatment of the suspected stains the method proposed by Rezzonico may be followed. A *minute* fragment of the stain, fabric or scrapings, is placed on a slide, and carefully teased with needles [preferably of glass—S.], to loosen the small particles of coagulum which have been imprisoned by the fibres; then the preparation is moistened with a drop or two of the fluid chosen by the observer, gently covered with a cover-glass, and left to stand for half an hour, after which time it is again teased out and examined. Or the method of treatment proposed by Tourdes may be adopted. Tourdes wrote that the stain chosen for examination should be intact, well marked, and not disturbed by having been pulled about, washed, or scraped. A fragment of this should be placed on a slide, and moistened with a few drops of the chosen fluid, the preparation being then allowed to undergo maceration for two or three hours—not more, lest the erythrocytes should become too much altered. Old stains, however, he noted, require maceration for one or two days, and in their case the preparation should be made on a hollow-ground slide, and covered with a cover-glass to form a damp chamber. The fabric, when sufficiently macerated, should be teased with glass needles, to detach the insoluble portions of the stain from the fibres. The examination should be very carefully and patiently carried out, all the fibres, with the

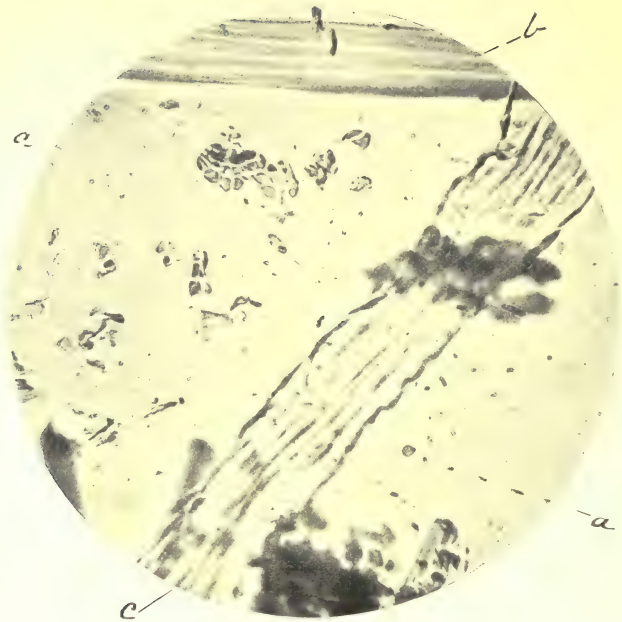

FIG. 3.

PREPARATION OF A MAMMALIAN BLOOD-STAIN IN MARX' FLUID.

× 350. Blood-stain on woman's undervest of silk and wool. *a, a*, Single erythrocytes; *b*, fibre of silk; *c*, fibre of wool.

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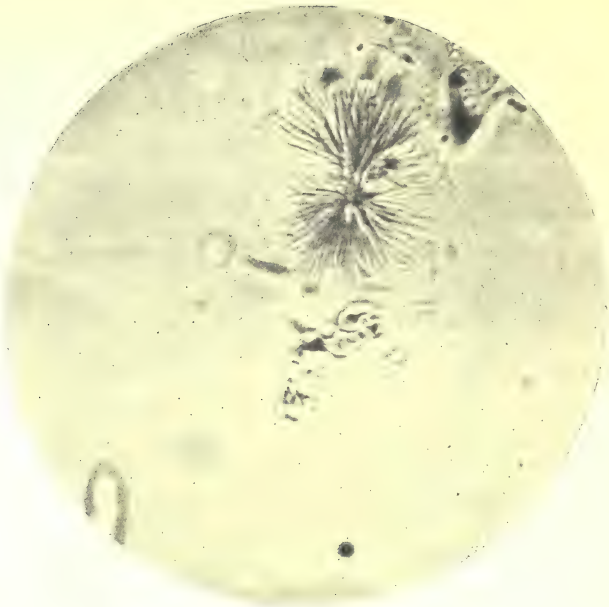

FIG. 4.

PREPARATION OF NON-MAMMALIAN BLOOD-STAIN IN MARX' FLUID.

× 500. Blood-stain caused by chicken's blood on cotton cloth, of which part of a fibre is seen at the edge of left lower quadrant. The feathery crystal bundle is due to the ingredients of the fluid. Nuclei of elliptical erythrocytes fairly distinct.

To face p. 51.

liquid that adheres to them, being examined, those which appear to be most coloured being probably the most fruitful of a positive result. He stated that it is of no use to look for erythrocytes of the appearance presented by those in fresh preparations of blood. 'What will be found after careful search will be rounded bodies, more or less spherical or flattened, and of a more or less deep tint, mixed with amorphous débris, the result of the destruction of other erythrocytes. The erythrocytes found are to be counted and measured, their form and colour and the difference between their centre and periphery carefully noted. As a contributory sign the presence of leucocytes is to be looked for. With a few erythrocytes, perfectly defined, and of a mean diameter of 0·0076 to 0·0083 mm., one has all that one can have for diagnosis, and the great probability is that the blood is human.'

Richter writes that when the fragment of the stain is treated with Pacini's fluid, weak caustic soda solution, pepsin-glycerin formalin, or physiological salt solution [he uses 0·8 per cent.—S.], and the preparation is examined with a power of × 60-400, isolated erythrocytes are never seen; but with human and other mammalian blood before us we find, pressed together, pale yellow non-nucleated circular or polygonal plates, which are easily seen in the thin edges of the masses in the field of vision, which masses are composed of fibrin, and coagulated, yellow, moistened, albuminous matter, with erythrocytes and leucocytes, the latter being rendered more distinct by the addition of acetic acid to the preparation, which, however, causes the contour of the erythrocytes to disappear. Where nucleated oval erythrocytes are present, a 1 to 5 per cent. solution of acetic acid should always be added, as by this manœuvre we are enabled to see clearly the small, closely-packed-together, glistening, round or oval nuclei, which of themselves give proof of the presence of blood. He notes that the mere finding of the blood-masses in the field is sufficient to lead the practised observer to diagnose 'blood'; but he insists that a spectroscopic examination of the stains should also be carried out. Marx writes that the fluid proposed by him is of service in

that it does not stain rust particles, and does stain and bring out clearly the contour of the cells in the masses. He recommends a high power for the examination.

Willcox, in a private communication, writes: 'If on clothing, a minute portion of the stain is removed and placed in 0·75 per cent. salt solution in a covered watch-glass, the watch-glass being placed in the incubator at 35° C. for about fifteen minutes. The stained cloth is then transferred to a microscopic slide, and carefully teased in some of the salt solution, a cover-slip applied, and the specimen examined for blood-corpuscles. The size of the blood-corpuscles seen should be carefully measured by the micrometer. It is noted whether the corpuscles have the characters of mammalian corpuscles. Another teased specimen is similarly made on an albuminized slide. This specimen is carefully dried at 35° C. It is then fixed and stained with Leishman's stain, or else it is fixed and then stained with hæmatoxylin and eosin. This procedure will show up the nuclei in the red blood-corpuscles of birds and reptiles, and prevent the possibility of this kind of blood being mistaken for mammalian blood. If the stain is on a weapon, and not on clothing, the same procedure is applied, except that a scraping of the stain is taken and placed at once on a microscope-slide, salt solution added, and, after soaking and teasing, the specimen is examined as described.'

Personally, I have used Hofmann's, Vibert's, and Marx' fluids, and am inclined to recommend the last named. The preparation may be made as recommended by Rezzonico. The less fluid used the better, I think, and it is of advantage to vaseline the edges of the cover-glass before putting it on the preparation. Thus any particles which might otherwise be forced out and lost by the fluid being squeezed between the cover-glass and the slide, will be retained and available for examination. The use of an albuminized slide does not appear to offer any special advantage.

The reader may think that in any given case he could at once distinguish between mammalian and non-mammalian erythrocytes in a stain. A few trials will go far to convince

him that a considerable amount of practice is required before he can do this with that certainty which the distinction in a forensic case demands. Much depends on skill in microscopic work, no doubt, but a great deal more depends on the treatment of the stain. And it must be remembered that, although we know that with the exception of the camel and the llama, all mammals have circular erythrocytes, and that all mammals' erythrocytes are, as a rule, non-nucleated, while, on the other hand, birds, reptiles, and fishes have elliptical nucleated erythrocytes, we *may* have nuclei in a few of the mammalian erythrocytes in the field; and, as Morache first pointed out, stains that are *over three weeks old* may give us mammalian erythrocytes which contain nucleus-like granules, or non-mammalian erythrocytes which show only highly-refractile granules, the remains of their nuclei.

Having fixed the fact that the blood before us is mammalian, we have to consider whether it is possible, by careful measurement of the erythrocytes seen, to determine their source.

First let us consider the measurements of human erythrocytes which have been made since Leeuwenhoek, with the poor microscope at his command, first measured them. The following table I have compiled mainly from Woodward, who reduced the vulgar fractions of a Paris, or English, inch given by Mandl in his account to decimal fractions of a millimetre. The discrepancies noted are striking.

Year.	Observer.	Measurement (mm.).	Year.	Observer.	Measurement (mm.).
1673	Leeuwenhoek	·00902	1826	Edwards	·00814
1720	Leeuwenhoek	·01327	1827	Hodgkin	·00902
1717	Jurin	·00789	1827	Wollaston	·00525
1724	Tabor	·00723	1830	Weber	·00525
1749	Senac	·0082	1834	Müller	·00525-·00902
1751	Muys	·01128	1836	Schultz	·00667-·00832
1760	Weiss	·01085	1838	Wagner	·00645-·00752
1763	della Torre	·00301	1842	Mandl	·008
1789	Blumenbach	·00789	—	Gulliver	·00794
1804	Villar	·00564	1848	C. Schmidt	·0077
1810	Sprengel	·00902	1858	Ch. Robin	·00751
1818	Bauer and Home	·01504	1863	Welcker	·00774
1819	Kater	·00677	—	Harting	·0073
1819	Young	·00451	—	Valentin	·0074
1821	Rudolphi	·00902	1866	Flint	·00726
1821	Prevost and Dumas	·00705			

And we must remember that these measurements were made by the foremost microscopists of their day, so that the difficulty of measurement in the case of fresh blood is apparent. How much greater this must be in the case of dried blood-stains the reader can easily imagine.

From the following table, which I have compiled from various sources, the reader will observe (1) that the same discrepancies exist when the erythrocytes of the domestic animals are measured, and (2) that some of these erythrocytes have measurements scarcely to be distinguished from those of human erythrocytes (see table on p. 55).

To remove the difficulty experienced by micrometrists (who recognized that in each species there is a difference between the maximum and minimum measurements obtained) in fixing the source of the blood examined, various methods have been devised.

Bethe proposed the construction of curves, whose abscissæ show the various sizes observed, while their ordinates show the number of erythrocytes found of each size. He himself admitted that the curves of human blood and of guinea-pig's blood were identical, and on reading his paper one will be struck by the predominance of the personal equation in his measurements. Birchmore has recommended similar curves, in which he has much confidence apparently, for he considers that they may be employed to determine the source of bloods from different individuals of the same species. Bell has given some examples of these curves, which appear to me to be very neat, but by no means reliable for forensic work.

Woodward recommended that the erythrocytes be observed under a $\times 3{,}700$ power, and Formad gives beautiful pictures of perfect corpuscles of the various animals from man downwards, magnified $\times 9{,}000$. Axtell had intended to show on a screen projection-images of various blood-preparations in a case in which he was consulted, so that the jury might appreciate the differences between the various erythrocytes, and see how clearly those of man may be distinguished from others.

Richardson wrote voluminously on micrometry and its

MEASUREMENTS OF THE ERYTHROCYTES OF MAN AND VARIOUS DOMESTIC ANIMALS—GIVEN BY DIFFERENT OBSERVERS.

Animal.	Gulliver.	Schmidt's figures.		Société de médecine légale.*	Tourdes.	Masson.	Dragendorff.	Däubler.	Malinin.	
	Reduced to mm.	Range.	Average.						Fresh blood.	Blood-stain treated with KOH.
Man ...	·0079	·0074-·008	·0077	·0075	·0074-·008	·0078	·0077	·0078-·009	·0077	·007-·0075
Dog ...	·0072	·0066-·0074	·007	·0073	·0066-·0074	·0071	·007	·007-·009	·007	·0062-·0065
Rabbit ...	·007	·006-·007	·0064	·0069	·006-·007	·007	·0064	·0065-·0081	—	—
Rat	·0068	·006-·0068	·0064	—	—	—	—	—	·0064	—
Cat	·0058	·0053-·006	·0056	·0065	·0053-·0056	·0057	·0056	—	·0056	—
Horse ...	·0054	·0053-·006	·0057	·0056	·0055	—	·0057	—	·0057	·0052
Ox	·006	·0054-·0062	·0058	·0056	·0056-·006	·006	·0058	—	·0058	·0054
Sheep ...	·0055	·004-·0048	·0045	·005	·0047-·005	—	·0045	—	·0045	·0041
Goat ...	—	—	—	·006	·006-·0065	·0062	·0062	—	·004	·0033
Pig	·006	·006-·0065	·0062	·0046	·004-·0046	—	—	—	·0062	·0055
Mouse ...	·0067	·0058-·0065	·0061	—	—	—	—	—	—	—
Buffalo ...	—	—	—	—	—	—	—	—	—	·0046

* The members of the committee were Mialhe, Mayet, Lefort, and Cornil. They drew up an 'Instruction,' in which the use of an ocular micrometer is recommended, to which Vibert took exception, as being likely to give very inaccurate results.

advantages, but he held peculiar views as to the way in which facts should be treated. In a private communication to Woodward he wrote that he 'was aware of the impossibility of distinguishing the blood of a man from that of a monkey or dog, but refrained from giving prominence to these facts, lest an improper use should be made of them in the defence of criminals'! Fortunately truth has but few such friends as Richardson was in the ranks of scientific men. It is worthy of note that at the time that he received this communication Woodward was not so firm a believer in the forensic medical merits of micrometry as he afterwards became.

Of the differential diagnostic value of the method Virchow wrote in 1857: 'I can but join Brücke in his condemnation of the method, and I do not believe that any microscopist will ever hold himself to be justified in setting a man's life in jeopardy on the strength of an uncertain determination of the drying coefficient of an erythrocyte.' Yet Virchow published Malinin's article in 1875. In 1865 Roussin observed that the dryness or humidity to which the erythrocytes in a blood-stain have been exposed, and the varying degrees of rapidity with which, when wetted, they absorb moisture, are stumbling-blocks in the path of the observer.

In 1874 Rabuteau (I have unfortunately mislaid the reference) protested against the differential diagnosis of mammalian blood by micrometry, since a difference of 0·0002 mm., such as existed—according to the 'Instruction' which a committee of the Société de Médecine Légale had drawn up—between the diameters of human and dog's erythrocytes is too small to be seriously taken to be the basis of a medico-legal opinion; and in 1885 Masson wrote: 'Our experience has shown that in the case of human blood its differentiation from the blood of a pig, ox, or cat is easy, from the blood of a dog difficult, from the blood of a rabbit uncertain, and from the blood of a guinea-pig impossible.'

Formad, however, was of the opinion that, 'if the average diameter of blood-corpuscles in fresh blood is less than

$\frac{1}{4000}$ inch [0·00635 mm.], then it cannot possibly be human blood; if the diameter is more than $\frac{1}{3500}$ inch [0·00725 mm.], then it may be human blood. If the blood-corpuscles, after exhaustive measurement, give a mean diameter of $\frac{1}{3300}$ inch [0·00769 mm.], then it is human blood (provided that it is not the blood of one of the wild beasts referred to). . . . And we have seen that blood can be diagnosed in its dried state and in blood-stains with the same certainty as fresh blood, provided the drying was rapid and perfect.' But, as Ewell pointed out in 1893, 'it has never been proven that dried corpuscles can be restored to their normal proportions. . . . It is impossible in the present state of science to say of a given specimen of blood, fresh or dry, more than that it is the blood of a mammal.'

Regarding the whole question of diagnosis by micrometry, Draper writes: 'We must remember the ordinary medico-legal conditions under which the expert works. (1) He has an old and dried stain of disputed origin. (2) The animals whose blood is normally invoked by the defence in disputing the contention of the prosecution are dogs, rabbits, pigs, oxen or cows, horses, and sheep or goats, and they are uncomfortably close to the human class in any scale of measurements of their blood-disks. (3) The animals whose blood is most in contrast with human blood in the matter of human blood-disk measurements, as the elephant, the leopard, the ibex, and the deer, are not likely to be cited at capital trials. (4) The action of fluids and solvents used to restore dried blood to something like its fresh state is attended with uncertainty in the matter of the uniformity of the results. There is no fixed standard. The great number and variety of these reagents prove this uncertainty. (5) Even under normal conditions in adult life the blood-globules of the same species, whether human or of the lower animals, do not have a uniform diameter, but show a considerable range between minimum and maximum. (6) Finally, the possible alteration in the size of the corpuscles, due not only to time and exposure, but also to diseased conditions.'

I have nothing to add to Draper's terse account of the

defects of micrometry as a means of differential diagnosis of mammalian blood.

Yet micrometry has been relied upon in forensic cases, in accordance with the state of knowledge at the time.

11. In 1858 Ch. Robin and Salmon were asked to examine a cotton blouse, and to state whether the stains on it had been produced by human or duck's blood. They treated the stains with Bourgogne's 'fluid No. 4a,' a secret preparation of whose composition I have been unable to find an account, and in the stains discovered erythrocytes which they stated 'had all a breadth of six to seven thousandths of a millimetre, seldom a little more, which is the normal diameter of the globules of [human] blood.' Further, they wrote: 'The elements of the blood forming the stains on the blouse are the elements of human blood. There is fibrin which, like that of human blood, is fibrillated, and reacts with acetic acid, etc. There are white corpuscles which have the volume, form, granulations, nuclei, and chemical reactions of the white corpuscles of human blood. There are red corpuscles, whose size, and circular, flattened, biconcave shape, and yellowish-pink colour are those of human red corpuscles, viewed by transmitted light by means of a microscope. These are dissolved, as are human red corpuscles, by water and by acetic acid, leaving no trace of a nucleus. But in the present state of science it is impossible to say more; it is impossible, from an examination of this blood, to fix the age or sex of the person from whom it was derived.'—*Ann. d'hygiène*, 1857, p. 368.

It is evident that this blood was mammalian, but it is not proved that it was human.

12. Malinin in 1875 reported the case of two nobles, who were arrested because in a stable which belonged to them there was found a board on which were stains which were suspected to be due to human blood. They stated that these were due to sheep's and goat's blood, and when—in the presence of eighteen confrères, as he remarks with satisfaction—Malinin examined the stains, he came to the conclusion that they were due to sheep's and goat's blood. Had he, as he

notes, confined himself to saying that the stains were due to mammalian blood, the two men would have been sentenced to imprisonment for life. So much confidence had he in himself—and in his fluid!—*Arch. f. path. Anat.*, 1875, 65, p. 528.

13. MARISCAL Y GARCIA was asked to examine some stains which were suspected to be due to human blood, and came to the conclusion that they were not due to human blood, on the ground that the circular erythrocytes found by him were only 0·006 mm. in diameter.—*Siglo médico*, 1889, 36, p. 311.

14. GRIGORESCU reported that, in a case in which he was consulted, he had determined the degree of desiccation of the erythrocytes in some blood-stains, and to their diameter had added '1 or 2 microns,' and found that they were human erythrocytes. He, however, asked that his conclusions might be controlled, and Babes, who was requested to examine the stains, declined to say more than that they were due to mammalian blood. The other evidence in the case showed that the stains were due to human blood, and Grigorescu took this to be a proof of the correctness of his method of diagnosis.—*C. r. soc. de biologie*, 1892, p. 325.

Firstly, I would remark that Grigorescu should have published to the world how he managed to solve the *crux* of the drying coefficient of erythrocytes; secondly, that he must have known that in a case like this it made a vast difference whether—admitting that he had solved this question—he added 1 or 2 microns to the diameter of the erythrocytes observed; thirdly, that Babes was right in confining himself to scientifically demonstrable facts.

Stains caused by Insects.—The French and German authorities have drawn attention to the similarity between the stains left by the crushing of blood-sucking insects and blood-stains—a matter which is not particularly noticed in any publication in the English language to which I have had access, although it is obviously one of some importance, in tropical countries especially.

In 1830 Chevallier was consulted in a case in which there were found on a man's shirt-sleeves some stains which he

alleged were due to bugs, but which were suspected to have been caused during the commission of a murder of which he was accused. After careful examination of the stains left by crushed bugs, Chevallier stated that he could find no difference between these and blood-stains, save that a bug-stain gave a solution which became turbid when chlorine was fused into it, and which, when treated with sulphuric acid, gave an aromatic odour.

Schmidt adversely criticized Chevallier's experiments, and gave the following as being the chief points of difference:

	Blood-Stain.	Flea-Stain.	Bug-Stain.
1. Colour of stain	Reddish-brown	Brownish-red	Brownish-red
2. Form	Rounded	With points all round it	Circular, 1-3 mm. diameter
3. When viewed against candle light	Cochineal-red	Brownish-red	Brownish-red
4. Surface	Rough, rarely with elevations in the middle	Rough, with thick elevations in the middle, which glisten	Smooth; no elevations in middle. Often several stains arranged garland-wise

Vibert stated that flea-stains are 0·5 to 3 mm. in diameter, and oval or round, but never with a pear-stalk point such as is found in a blood-spirt, and that they often yield the spectrum of blood and crystals of hæmatin chloride.

Ch. Robin describes them thus: 'They are composed of masses of a homogeneous, amorphous, transparent, colourless substance, which swells up and tends to become disintegrated or dissolved on the addition of water. Imbedded in these masses lie granules, to which they owe their colour, and which lie close together, forming the greater part of the masses. These granules are of a brownish-yellow colour, some with a reddish and others with a greenish sheen, highly refractile, being more brilliant at their centre than at their circumference, like fatty substances, which they also resemble in being insoluble in acetic acid, and nearly wholly

soluble in hot alcohol or in ether. Along with them are found needle-like crystals, whose composition is as yet undetermined.' Vibert, however, stated that this description does not always hold good, and that he believed that no difference is apparent in many cases between a flea-stain and a stain due to pure blood.

Robin described bug-stains thus: 'The dust-like excrement is formed of little dried pellets, 1 to 10 μ in diameter, spherical or ovoid in form, reddish-brown in colour, and brighter in hue at the centre than at the circumference. These may lie separated from one another or massed together. There are also crystals, which resemble those of organic substances, and have the form of feathered lozenges, needles, and sometimes prisms.' Legrand du Saulle and Vibert both accept this description as correct.

Schauenstein found that from the stains made by blood-sucking insects, especially by fleas, there may often be obtained crystals of hæmatin chloride, while the stains, as shown by the microscope, contain erythrocytes, and this observation is confirmed by the work of Janeček, who found that hæmatin chloride may be obtained from the excreta of bugs and fleas, and that the excreta of the common house-fly yield a large quantity of the crystals, and also a distinct spectrum of hæmatin or hæmochromogen, according to the reagents used.

Hofmann thought that a mistake could hardly occur, on account of the known forms of insect-stains; but Brouardel and Vulpian, in a case in which they were consulted, found some flea-stains on a man's shirt that were, when viewed with a magnifying-glass, very like the stains which they produced experimentally by sprinkling the shirt with blood; and, as we shall see, Biondi obtained the precipitin reaction from stains produced by the crushing of blood-laden fleas, bugs, and mosquitoes.

I conceive it to be very likely that, if only a few droplets of blood be present on the clothing of an accused person—droplets such as may be projected by the breaking of the blood-bubbles made by a man whose throat is cut—the

allegation of the defence would be that these droplets were due to insect-stains. It is therefore of service to go further into the question of the differentiation of insect-stains. From the experiments which he carried out Schöfer concluded that the crystals, whose presence in a bug-stain was first noted by Robin, are of uric acid, and that a mistake might occur if a stain that is due to some substance other than blood has been fouled by insect excreta. He confirmed the observation of Hofmann that in insect-stains portions of the insects, as well as their eggs, are often to be found. When he crushed a blood-laden bug on cloth, and wiped the crushed mass off the fabric, he found in the preparation of the stain portions of the tracheæ and bristles of the insect. A bug's bristles are characteristic, being yellow, trebly serrated at their free end, with the shaft like the deplumated shaft of a feather (see plate). In a case in which he examined the neck-piece of a shirt that had been much stained by lice he obtained crystals of hæmatin chloride and uric acid, and found the singly-pointed bristles which are characteristic of the *Pediculus corporis*, and a maxilla of this insect.

I have carried out a number of experiments by crushing blood-laden bugs on underclothing, and have found that it is not always possible to find the tracheæ or bristles; indeed in about 10 per cent. of the preparations I was unable to find them. The preparations contained all the stained material in each case, so that if the bristles were present they must have been found. I would urge that others make the further observations that are required on this point. In order that my experiments may be justly criticized, I may say that in each case the fabric on which the bug had been crushed was lightly swept with the finger to remove the crushed mass; then *all* the fibres of the stain were carefully teased out, the entire stained portion of the fabric being treated on one slide. The preparation was then treated with Hofmann's, Vibert's, or Marx' fluid, or with a solution composed of saturated solution of caustic soda, 1 part to 2 parts of 0·85 per cent. salt solution, and covered with the largest cover-glass that I could procure, the edges of which had been vaselined. Each

FIG. 5.

BRISTLE FROM A BUG-STAIN.

× 500. *a*, A cotton fibre out of focus.

To face p. 62.

preparation was then searched for half an hour. Considering the large number of bristles that the bug has, it is matter for wonder that these are not to be found in every preparation. The blood-masses were easily enough detected, but in the great majority of cases anything approaching a single well-formed erythrocyte was not to be seen.

So much for the detection of blood-stains by means of observation of the cell elements of the blood, a means of which Cauvet made use in the three cases published by him, to the exclusion of all other means save the Teichmann test, while Mariscal y Garcia appears to have relied on it alone in the case published by him.

Before proceeding to consider whether any other information can be obtained by the microscopical examination of a stain, we may note as a curiosity that Cotton believes that the changes undergone by the erythrocytes during the process of drying may aid us to differentiate their source. He states that human and equine erythrocytes become crenulated, while those of the guinea-pig contract in a rosette form, and those of the sheep become rugose over their entire surface. He also states that in all species the erythrocytes of the female tend to lie in rouleaux in the field, while those of the male lie scattered about. With every desire to be respectful to Cotton, I would suggest that his keen desire to find a solution of the problem of the differentiation of blood from different species by means of the microscope has influenced these observations, which are reported by him in the *Bulletin général de thérapeutique*, 1901, 141, p. 334.

Long ago Kölliker had noted that the crystals of hæmoglobin vary in different species, and Bojanowski, and also Misuraca, considered that this fact might be of use in forensic practice, while Copeman stated that crystals of hæmoglobin could be obtained only from the blood of man and monkeys.

Dvornitschenko published illustrations of the various forms of crystals found in the bloods of different species, but latterly he came to the conclusion that Misuraca's and Copeman's methods are of no use when one has to deal with blood-stains. Michelsson went carefully into this question, dealing

with stains caused by the blood of man, the ox, horse, sheep, pig, dog, rabbit, hare, goose, and domestic fowl, on silk, wool, cotton, linen, soft and hard wood, paper, glass, and metal, the stains being from a few days to five years old. He found that the forms of the hæmoglobin crystals are of no use in forensic medicine, for they vary with the age of the stain, cannot always be obtained from fresh stains, and are rarely obtainable from stains that are over two months old, while, when crystals are present, the non-typical forms may be so numerous that one cannot tell from what species the blood has come.

Neumann (and Day also) believed that the pattern formed by the minute cracks seen on the surface of a dried blood-stain indicates the source of the blood, but no one else seems to have thought this point worthy of study. Magnanimi thought that the resistance offered by the blood-pigment to alkalis, a resistance which varies in different species, might be taken to be an indication of the source of the blood. Ziemke (332) confirmed the fact that the resistance to the action of alkalis does vary in different species, but the test is of no practical value.

At one time Ehrlich was inclined to believe that neutrophile granules are characteristic of human leucocytes, and with this opinion Corin agreed; but later Tamassia (276), Ilberg, and Hirschfeld reported that neutrophile granules are not peculiar to human leucocytes. Hirschfeld believes that in fresh blood the size and arrangement of the granules, and their power of taking a stain, might serve to distinguish human from other blood, but that in the case of a dried blood-stain this distinction cannot be made.

Husson thought that, where the microscope and spectroscope fail to yield positive evidence of blood in a stain, 'it may be of interest to show the trouble that the accused person has taken to wash one part of an article of clothing rather than another part. Water, as a rule, does not suffice to remove all traces of blood; the stain must be soaped, and if this be not done with a large quantity of water, it rarely happens that the material does not retain some trace of soap,

which may be demonstrated without interfering with the search for crystals of hæmatin chloride. A piece of the material, cut off at the suspected part, is put into a watch-glass and moistened with a few drops of distilled water. The preparation is then kept at 40° C. for two hours in the sand-bath, and to prevent its becoming desiccated, a drop of water is added to it three or four times during the maceration. The material is then squeezed with forceps, and the expressed liquid will by its colour show whether one may hope to obtain crystals of hæmatin chloride from it. It is evaporated drop by drop on a slide, and, if the residue be too much spread over the surface of the slide, it is scraped off and collected in the middle, and fixed there by the addition of a drop of a 1 per cent. solution of iodide or chloride of potassium. The material is then placed in a watch-glass and treated with a little glacial acetic acid, which is squeezed out of it and evaporated on the first residue, the preparation being then covered with a cover-glass, under which a drop or two of glacial acetic acid is allowed to run. It is then heated till it boils, and set apart to cool, being slightly tilted, so that any liquid which remains may be collected at one part. If hæmatin is present in the stain, we shall obtain the crystals of hæmatin chloride. If soap be present, we shall have yellow droplets of oleic acid, and the characteristic needles of margaric acid, which are never straight, being more or less curved like elongated C's or commas. If much soap is present, the needles will lie together, forming tree-like masses; if, on the other hand, they are few in number, they will lie in pairs, or in small bundles, which are somewhat like fibrin lying on tissue débris. It is specially because of this resemblance that I bring this paper before the Académie [des Sciences], for the similarity of form, although it is not close, might lead to mistakes being made. A few filaments of fibrin seen under the microscope, without any crystals or stars of hæmatin chloride, do not appear to me to be enough to prove the presence of blood [nor to anyone else nowadays, I believe—S]. When the washed stain is large enough for a control test to be made, another fragment of the material

may be cut off and boiled with distilled water. . . . The liquid is then filtered through a very small filter, evaporated, and the residue calcined in a platinum capsule. It is then dissolved in a drop of distilled water. If this blues red litmus-paper, it shows that the alkali of the soap is present, as well as its acids.'

Obviously this test must be carried out with at least one control from an unsuspected portion of the garment.

The following cases, which I have culled from the literature, are given as examples of what the microscope can do for us in the matter of blood-stains in forensic practice:

15. Roussin.—A blue blouse and a handkerchief, both stained with blood, were found in the house of a man who was suspected of having committed a murder. In the stains there were found elliptical erythrocytes and three fish-scales. Roussin stated that the stains were certainly not due to human blood, but to the blood of a bird, a reptile, or a fish—probably a fish.—*Ann. d'hygiène*, 1865, 23, p. 139.

16. Roussin and Tardieu.—A man accused his discarded mistress of having lured him to her apartment, and of having, while manually caressing him, rendered him incapable of being faithless to her by amputating his penis. On a petticoat that was found in the room were found some stains, which the woman stated had been caused by the blood of a goose that she had killed. No elliptical erythrocytes were found in the stains, but many circular ones, and the experts stated that, in their opinion, the stains were certainly not due to the blood of a bird, but they could not say for certain that they had been caused by human blood. —*Loc. cit.*

17. Frigerio.—In some yellow stains that were found on the trousers of a man who was suspected of complicity in a murder there were detected strands of silk arranged like those of the first involucre of a cocoon, and many Cornalia's corpuscles. The conclusion arrived at was that the stains had been caused by silkworms which were affected with the pebrina parasite.—*Gazz. med. ital. lombarda*, 1884, pp. 323, 333.

18. SCHÖFER.—On the outer surface of the flannel drawers of a man who was accused of having committed a murder were found three stains, which he stated were due to his having crushed a bug. The stains yielded crystal of hæmatin chloride, and—on treatment with modified Pacini's fluid—erythrocytes whose diameter was 0·007 to 0·0078 mm., 'which is nearly that of human erythrocytes.' There were also found two tracheæ of an insect and two bristles, one entire and the other broken, which resembled the bristles of *Cimex lecticarius*, and were unlike the bristles of any other blood-sucking insect. The conclusion arrived at was that the stains had been caused in the manner stated by the accused person.—*Wiener klin. Woch.*, 1893, p. 643.

19. WOOD.—A man was accused of having committed a murder in a barn, and of having chopped off the arms and legs of his victim there. The blood-stains that were found in the barn were, he alleged, due to his having bled a horse, and the jury found him not guilty on the ground that it was impossible to distinguish between human and equine blood. Wood was asked to examine some of the stains, and in one he found a fragment of bone, with muscular tissue attached to it; but as this evidence was brought forward late in the trial, it was ruled to be inadmissible.—*Boston Med. and Surg. Journ.*, 1901, p. 533.

20. FLORENCE.—On a coat which belonged to an accused person there were found some stains which looked like washed-out blood-stains, and which he stated were due to hare's blood. On careful examination there was found on the inside of one of the seams of the coat a punctiform blood-stain, in which, on microscopical examination, there were found elliptical erythrocytes, fragments of duck's down, fibres of cotton, and débris of green algæ. Florence was of opinion that the stains were due to duck's blood, and that, as avian blood coagulates at once, they had dried on the surface of the fabric of the coat, and thus could be wiped off with a damp cloth without the colouring matter penetrating the fabric. He concluded, too, that they had been wiped off with a handkerchief that had been wetted by being dipped

into a horse-pond; for the coat was not of cotton, and the horse-ponds in the neighbourhood of Charolles were full of green algæ.—*Arch. d'anthrop. crim.*, 1901, p. 255.

21. HAUSER.—A man was accused of having satisfied his passions with a goose. He alleged that the blood-stains on his trousers were due to his having enjoyed the favours of a menstruous woman; but the stains contained elliptical nucleated erythrocytes, so the case was clear.—*Münchener med. Woch.*, 1904, p. 289.

22. A. ROBIN.—Some blood-stains that were found in a fowl-house were found to be composed of avian erythrocytes, and thus it was clear that the murder in question had not been committed in the fowl-house, which was near the barn in which the corpse was found.—*New York Med. Journ.*, 1904, pp. 433, 500.

23. DRAPER.—In a murder case which occurred at Goron, Mass., the accused person stated that the stains on his clothing were due to fowl's blood, but they were found to contain no elliptical nucleated erythrocytes.—'Text-book of Legal Medicine,' *Philadelphia*, 1905, p. 440.

24. UHLENHUTH.—The driver of a cart was found lying dead in a pool of blood on the highway. On a sack that lay on the seat of his cart were found some stains, and the question arose as to whether he had been murdered. On examination of the stains on the sack these were found to be due to blood, and to contain avian erythrocytes, so it was clear that the man had not been knocked off his seat.—*Deutsche med. Woch.*, 1906, p. 1244.

25. ERDMANN examined some earth which was supposed to be contaminated with blood, and found corpuscles which at first sight looked like erythrocytes, but on closer examination were seen to be cells of an alga—the *Porphyridium cruentum* Naegeli.—TOURDES, *art.* 'Sang (médecine légale),' in 'Dict. encycl. des sci. méd.,' *Paris*, 1878.

CHAPTER VI

SEROLOGICAL TESTS FOR BLOOD-STAINS: AGGLUTININS

When the blood-serum of an animal is allowed to act upon the *erythrocytes* of an animal of a not too closely related species, it causes these to become clumped together and dissolved by reason of the *agglutinins* and *hæmolysins* which it contains. If it be allowed to act on the *blood-serum* of the animal, it precipitates the precipitable substance of this serum by reason of the *precipitins* which it contains.

These three substances have been the subject of much study, and their action has been taken as the basis of forensic tests for the source of a blood-stain. These tests we shall now discuss in the order indicated above, but it is necessary briefly to study the various substances on whose action they are based.

Agglutinins.—In 1890 Landois observed that when the erythrocytes of an animal are brought into contact with the blood-serum of an animal of not too closely related species, they become agglutinated—clumped together. Then Landsteiner found that the serum of man agglutinates the erythrocytes of animals far removed from man, and also that the serum of many cases of severe disease in men has the power of causing the agglutination of the erythrocytes of other men —that iso-agglutinins as well as agglutinins exist in human serum. Donath found that iso-agglutinins are present in cases of chlorosis, and Lo Monacho and Panichi found them present in many cases of malaria, as did Ascoli, who also found them present in cases of stomach cancer, pneumonia, typhoid fever, and phthisis, and noted that they vary in amount in the same individual at different times. Their

existence has also been noted by v. Decastello and Sturli. Ascoli also noted that auto-agglutinins may be present, as did Mme. Girard-Mangin; but Biffi* doubted their existence, and proposed that as the basis of a test of guilt might be taken the presence or absence of agglutination when the erythrocytes of a person are treated with an extract of a blood-stain which is believed to have been caused by blood shed by him: if his erythrocytes are agglutinated, the stain cannot have come from him. Unfortunately, Marx and Ehrnrooth have observed that certain individuals' erythrocytes resist the action of iso-agglutinins, and that the iso-agglutinins cease to make their presence felt if the stain be more than a month old, so that Biffi's test, even if it were correctly based on the non-existence of auto-agglutinins, is quite worthless in practice.

A phenomenon so marked as is that of agglutination has naturally been the subjection of much discussion, but its true cause has not yet been definitely settled. Bordet believes that it is a purely physical process. Nicolle supposed that there exists on the surface of the erythrocytes a 'substance agglutinative,' which swells up and causes neighbouring erythrocytes to adhere to one another. Paltauf believes that the phenomenon is due to the attraction exercised on the erythrocytes by the particles of a precipitate which is formed. Gruber at first was inclined to postulate the existence of 'glabrificin' on the surface of the erythrocytes, but later has been led to believe that their protoplasm and enveloping membrane undergo a change, which in its turn causes the agglutination to take place. Myers believed that chemical changes take place in the erythrocytes, and that in consequence some proteid becomes insoluble or less soluble in the serum, and surface tension does the rest. Joos believes that the amount of salt in the serum plays the chief part in the production of the phenomenon: Bordet has shown that when agglutination has ceased, the separated cells cannot be reagglutinated unless there be present some salt in the

* 'Sobre las hemoaglutininas de la sangre humana,' *Bol. de la acad. nacional de med. de Lima*, 1903, No. 2 (Reprint).

surrounding medium; and in Joos's opinion this salt's action is active, in that it either becomes united to the erythrocytes themselves, and by thus modifying them renders them agglutinable, or enters the already formed union of erythrocyte and agglutinin. Friedberger believes that the salt is certainly of great importance, but he holds that its action is merely passive, consisting in the causation of a disturbance of the molecular equilibrium of the surrounding medium. Albrecht holds that the superficial or myelinogenous layer of the erythrocyte-protoplasm has its adhesiveness increased by the swelling and partial dissolution which it undergoes in the presence of salt, and that the erythrocytes, by reason of this swelling, come to lie more in contact with each other, while by the outstreaming of their dissolved products into the surrounding medium they become pressed together. Mme. Girard-Mangin believes that the fact that the erythrocytes are electro-negative (tending to collect at the positive pole) has nothing to do with agglutination, which is due to the amount of colloid present in the serum—the less the better—and to the fact that the erythrocytes are surrounded by a fluid into which have passed the salts that have escaped from them. Weidenreich appears to hold a similar opinion, while Capogrossi holds that a change occurs in the erythrocytes themselves, a change which he does not describe, however.

It may here be noted that Lewis, and also Weidenreich, hold that the normal form of the erythrocyte is that of a bell, and not that of a biconcave disk. The latter observer has seen this form in the mesenteric capillaries of living rabbits, and suggests the following as a means of demonstrating the truth of his contention: When blood is obtained from an animal it should be at once defibrinated and centrifugated, so that some serum may be obtained in a few minutes. Into this serum a drop of blood is introduced, and the preparation covered with a vaselined cover-slip, and observed under the microscope till hæmolysis begins. Or a drop of rabbit's serum may be placed on one's finger-tip, which is then pricked so that the blood droplet may exude into the serum,

and the mixture of blood and serum is at once put on a slide, and covered with a vaselined glass and observed.

Ruffer and Crendiropoulo, and also Figari, believe that it is the leucocytes that form the agglutinins, and Capogrossi holds that the spleen plays no part in their manufacture. This question is still open.

Landsteiner precipitated the globulins of the serum by adding to it distilled water containing a maximum of carbon dioxide, and found that while the precipitate had agglutinating power, the supernatant fluid had none.

Winterberg found that when serum is treated with globulin precipitants, its agglutinating power is lessened. We may conclude, then, that the globulins form the active agglutinating part of a serum.

Several observers have shown that not one agglutinin, acting on all erythrocytes, is present in a serum, but that the serum contains several agglutinins, each of which is specific for the erythrocytes of a certain species and its closely allied species; and it has been shown that when an animal A is immunized by the injection of the erythrocytes of species B, its serum contains a large quantity of the specific agglutinin for this species; so that, when the serum is diluted to a degree which so weakens the normal agglutinins present in the serum that these cease to act, this specific agglutinin is still present in sufficient strength to make its presence felt, when the dilute serum is brought in contact with the erythrocytes of species B.

As Bordet first showed, the agglutinin is not affected by the serum being heated to 56° C.

Agglutinin Test.—In 1904 Marx and Ehrnrooth reported that they had found that when the blood droplet obtained from a pin-prick of the observer's finger-tip is mixed with an extract of a blood-stain, obtained by soaking the stained material in 0·6 per cent. salt solution, the erythrocytes are agglutinated if the stain has been caused by the blood of a mammal not man, while they at most only form rouleaux if the blood be human, or become polygonal if the blood be simian.

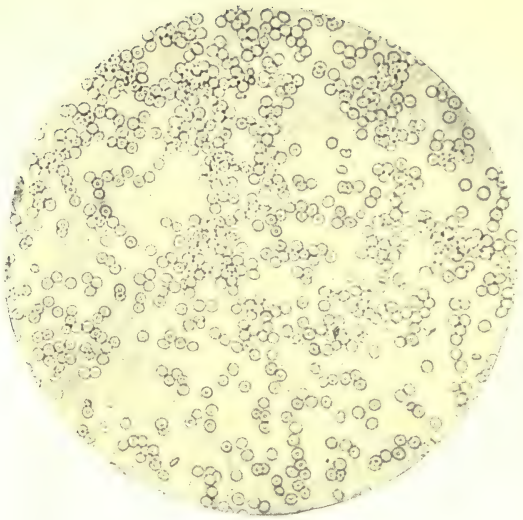

FIG. 6.

HUMAN ERYTHROCYTES (S.) UNAFFECTED BY A STRONG EXTRACT OF A STAIN DUE TO CANARY'S BLOOD.

Marx-Ehrnrooth Test.

To face p. 73.

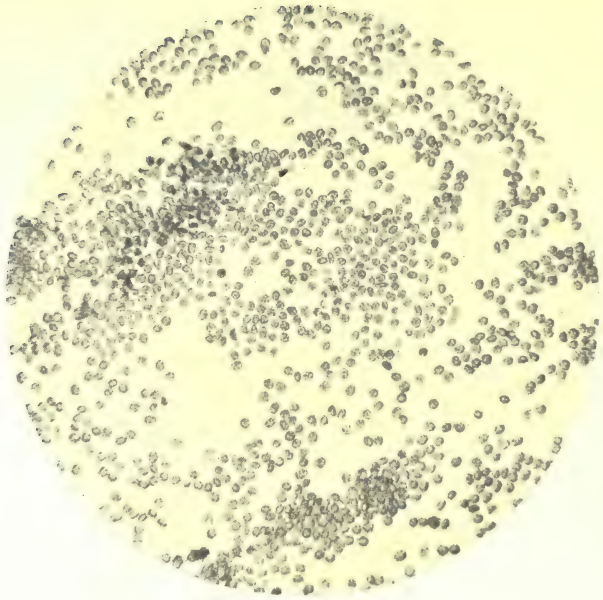

FIG. 7.

HUMAN ERYTHROCYTES (FRL. V. F.) AGGLUTINATED BY A STRONG EXTRACT OF A STAIN DUE TO RABBIT'S BLOOD.

Marx-Ehrnrooth Test.

As the rabbit is more akin to man than are the birds, the agglutination ought to have been marked in the case of the canary's blood. (See Fig. 6.)

To face p. 75.

The droplet of finger-blood, as small as possible, is mixed with a drop of the stain-extract, by stirring the two together for a few seconds with a glass rod, and then the preparation is observed under a low power. They state that, when the stain is due to human blood, the erythrocytes are always distinguishable one from the other; in the case of monkey's blood they lie as in the case of human blood, but instead of being mostly crenulated, may become shrunken and polygonal; in the case of other bloods they lie clumped together, the individual erythrocytes being no longer distinguishable, and lysis of the erythrocytes occurs.

Or the preparation may be made thus : A drop of the stain-extract having been conveyed to a slide, a cover-glass charged with a droplet of finger-blood is placed on this, and the edge of the preparation is examined.

In order to remove any interference with the test, which might be caused by the presence of iso-agglutinins in extracts of stains of less than a month old—which time they take to be the limit of the action of iso-agglutinins in dried stains—they recommend that, in case of doubt, there should be added to the preparation a drop of a fluid, obtained by mixing some of the observer's blood with four or five times its volume of 0·6 per cent. salt solution, and letting the mixture stand for twenty-four hours, the upper clear layer of fluid being then used. They state that the addition of a drop of this fluid will cause the rouleaux formed by the action of iso-agglutinins of homologous blood to become more marked, whereas the clumps formed by the action of agglutinins of heterologous blood became looser.

All that they claimed for the test was that it might be used as a preliminary test, before the precipitin test is carried out. They recommended that the tiro should begin by working with a 1 : 2 dilution of defibrinated blood, passing to more dilute solutions as he becomes familiar with the reaction.

Pfeiffer reported that he had obtained positive results when he tested blood-stains thirty-seven years old on wood and twenty-four years old on linen, and he was of opinion that as a preliminary test its value had been established. Carrara

found that heating the stains to 70° C. prevented the reaction, and de Domenicis reported that heating them to between 108° and 111° C. for a few minutes had this effect. He also noted that he had obtained the reaction with putrefied blood, six-months-old meconium stains, fresh albumin, lemon-juice, and grape-juice.

Florence, in his experiments on iso-agglutinins used water to extract the stains, so his results may be taken to have been by no means impeccable.

Martin made various experiments with the blood of man, horse, ass, ox, sheep, goat, deer, fallow-deer, roebuck, pig, wild-boar, bear, dog, cat, rabbit, hare, guinea-pig, mouse, mole, polecat, elephant, monkey, duck, pigeon, crow, screech-owl, domestic fowl, adder, and leech, and from their results came to the conclusion that the test is not to be depended upon, as its certainty is not that which forensic work requires.

I carried out several series of experiments, using in some a 0·85 per cent. and in some a 0·6 per cent. salt solution to extract the stains, the erythrocytes used as indicators being in some cases my own, and in others those of friends, who kindly placed their finger-blood at my disposal. The preparations were made as recommended by Marx and Ehrnrooth, or in a hanging-drop, as recommended by Carrara. The stains tested were due to normal human blood, menstrual blood, and to the blood of the monkey, dog, rabbit, cat, pig, mouse, and canary.

With my own erythrocytes I obtained agglutination only once, and this was with an extract of a menstrual blood-stain ; with the blood of others I obtained the reaction only once with a heterologous blood, the erythrocytes of Frl. v. F. being agglutinated by an extract of a rabbit's blood-stain. I consider the test to be of no value for forensic purposes.

CHAPTER VII

SEROLOGICAL TESTS FOR BLOOD-STAINS: HÆMOLYSINS

In 1869 Creite, at that time a student of medicine, observed that when an animal receives into its circulation a quantity of foreign blood, it presents certain well-defined signs of disease, chief among which is hæmoglobinuria—the passage of urine which contains blood-pigment. He noted, too, that when the animal's erythrocytes are brought into contact with the serum of the foreign blood they are dissolved, but he does not appear to have appreciated the connexion between this fact and the hæmoglobinuria. In 1875 Landois made many experiments with a view to find out why such disastrous results followed the transfusion of foreign blood. He observed the occurrence of hæmoglobinuria, and referred this to its true cause—hæmolysis; and he noted that these results did not follow the transfusion of a blood of a closely related species. Friedenthal has since then shown that, in the case of a closely related species, the whole of the foreign animal's blood may be allowed to pass into the circulation of the animal which is the subject of experiment, and thus to replace its blood, without an untoward result being observed.

In 1898 Belfanti and Carbone showed that hæmolysins may be artificially produced in the blood of an animal by immunizing it by means of injections of a foreign blood, and that these are specific for the erythrocytes of the species to which the animal whose blood has been injected belongs, and this observation has been confirmed by many observers,

who have written numerous articles on the subject of hæmolysins, a subject of which all is not yet known.

At present we know that the phenomenon of hæmolysis is produced by the united action on the erythrocytes of two substances, which exist in the hæmolytic serum. These substances are—(1) the *Complement* or alexine, which is present also in normal serum, is very labile, being so modified by the serum being heated to 56° C. that its action no longer occurs, and becoming changed to an inactive substance also if the fresh-drawn serum be allowed to stand for a few days, even if it be kept on ice in the dark; and (2) the *Amboceptor*, immune body, or substance sensibilisatrice, which, as the second name implies, is only present in an antiserum which has been produced by immunization. This is stable, resisting heat, and not tending to deteriorate by being kept.

An antiserum whose complement has been rendered inactive by heat may be re-activated by the addition of some fresh normal serum, whose complement takes the place of that which has lost its power.

Exactly how these two substances act has been, and probably for some time will continue to be, the subject of lively discussion. The German school of serologists accepts Ehrlich's explanation of the phenomenon of hæmolysis, which may briefly be stated thus: Every cell has a 'giant molecule' of protoplasm, comprising an 'active nucleus,' which carries on the special functions of the cell protoplasm, and 'side-chains' or 'receptors,' which anchor any albuminous substances that may come into contact with them, and fit them —the functions of the side-chains being to serve nutrition. In hæmolysis these receptors are represented by amboceptors, which have two groups of atoms—the cytophile, which are united to the receptors of the erythrocyte, and the complementophile, which are united to the haptophore group of atoms of the complement, whose zymotoxic group has thus an opportunity of acting upon the protoplasm of the erythrocyte. The cytophile group of the amboceptor is anchored *only* by the receptors of the erythrocytes of the species from

which the immunizing blood has been derived, or a closely related species, and the whole process of hæmolysis is a chemical one.

The French school, of which Bordet is the protagonist, scouts the idea that the substance sensibilisatrice (amboceptor) has a complementophile group of atoms. In its view the substance sensibilisatrice sensitises the protoplasm of the erythrocyte, and by so modifying it renders it capable of absorbing the alexine (complement), the process of hæmolysis being entirely physical.

Hæmolysis consists of the destruction of the erythrocytes, whose hæmoglobin passes out into the surrounding medium. The phenomenon always occurs when the erythrocytes of *any* animal are brought into contact with distilled water, the process here being physical and dependent upon the fact that the water is not isotonic—is anistonic—for the erythrocytes. But the hæmolysis which we have to consider is a lysis of certain erythrocytes, and no others, by a specific antiserum.

In 1902 Gengou showed that not only cells but also dissolved albuminous substances, when mixed with the antiserum to which immunization with them gives rise, have the power of absorbing alexine—of deviating complement. He considered that this fixation of alexine is due to the formation of a precipitate by the action of the precipitin of the antiserum on the precipitogen of the immunizing substance, and this view is accepted as correct by Gray. On the other hand, Neisser and Sachs, Muir and Martin, and also Friedberger, do not admit the necessity of a formation of a precipitate for the phenomenon of complement deviation to occur.

In 1905 Moreschi showed that very little of the immunizing substance is needed for the production of complement deviation by the complex of immunizing substance *plus* antiserum, and his results have been confirmed by Neisser and Sachs and by Muir and Martin.

The following diagrams may serve to make clear what is meant by the terms above employed:

We have in 1—*C* the complement with its zymotoxic atom group *Z*, and its haptophore group *H*; *A*, the amboceptor of the hæmolytic antiserum, with its complementophile *CO* and cytophile *CY* groups; and *E*, the erythrocyte, with its receptor *R*. In 2 *E* has anchored *A*, which by *CO* has anchored *C*, whose *Z* can now act on *E*. Hæmolysis will take place. In 3 we have A_2 the amboceptors of the antiserum which are anchored by *S*, the antigen (immunizing substance), and have been completed by *C*. There is thus no *C* available to complete the amboceptors *A* of the hæmolytic serum which have been anchored by the erythrocytes *E*. Complement deviation has taken place as a result of the mixture of antiserum and antigen, and hæmolysis cannot take place. These diagrams are, of course, founded on the famous diagrams of Ehrlich.

Hæmolysin Test.—In August, 1900, Deutsch read a paper at the International Medical Congress held at Paris, and gave the details of a test for blood which he had devised on the ground of the specificity of artificially-produced hæmolysins. For obtaining these hæmolysins he used blood, prepared thus: Placental blood, or blood obtained by venesection, was defibrinated and then kept on ice for twenty-four hours. Then the blood was centrifugated, and the amount of erythrocytes originally present in 10 c.c. of the blood was injected into the rabbit. The injections were repeated thrice, and seven days after the last injection the rabbit was bled, and the serum which separated out from the blood-clot was tested, and only that which was found to have—when diluted 1 : 4—a marked hæmolytic effect on human erythrocytes was used for the test.

The blood-stain which was to be examined as to the source of the blood was scraped, and the scrapings treated with phenol-salt solution (phenol 2 grammes, common salt 9 grammes, water 1,000 c.c.), and some of the extract pipetted off into a watch-glass, and there thoroughly mixed with the antiserum—3 parts of extract to 1 of antiserum—and some of this mixture was then sucked up into a capillary-tube of 2 mm. diameter, whose ends were then

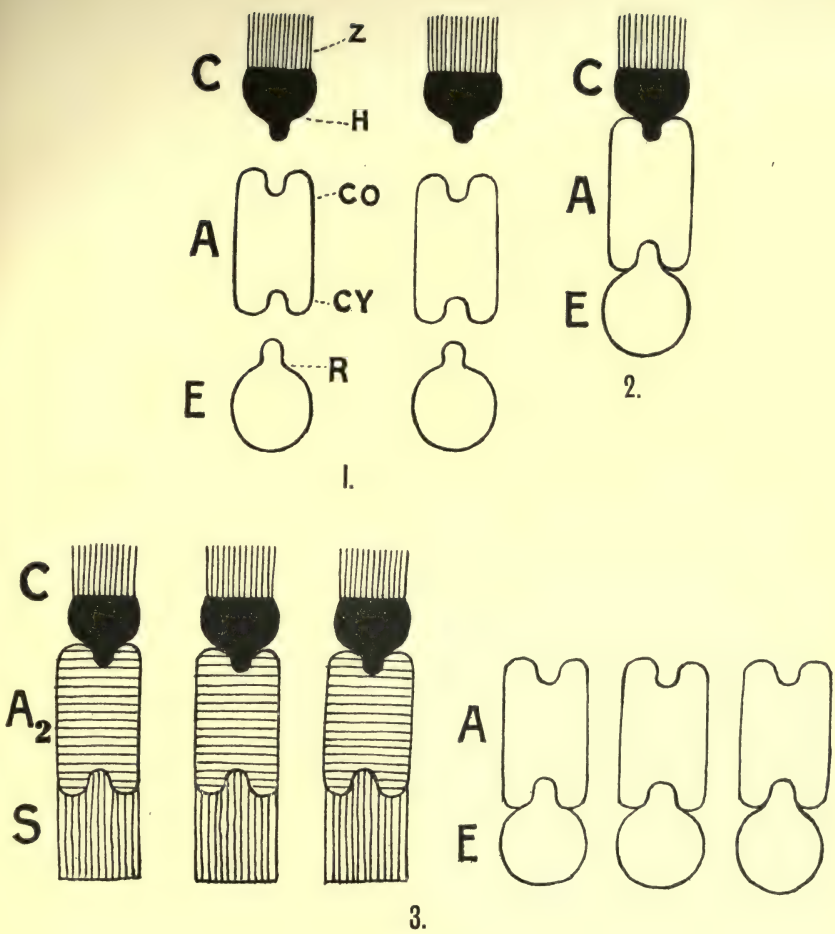

FIG. 8.

To face p. 73.

sealed in the flame. As controls were taken: (1) some of the extract alone; (2) some of the extract mixed with an antiserum which was known to be hæmolytic for the erythrocytes of the animal whose blood was alleged by the accused person to have caused the stain; and these controls and the test-tube were kept horizontal in the thermostat at 37° C. for twenty-four hours, and then examined. If in the test-tube hæmolysis was found to be complete, this was taken to be proof of the fact that the stain was due to human blood; if, on the other hand, it was but slightly advanced in the test-tube, and markedly advanced in the second control-tube, this was taken to be proof of the truth of the allegations of the accused person. The first control-tube was a test of the non-occurrence of hæmolysis in the extract alone.

Where only a small blood-stain was available for examination Deutsch recommended that a fibre or two of the stained material be treated with the phenol-salt solution, and the test carried out in a hanging-drop preparation.

He pointed out that the test was interfered with when the albumin of the blood had been coagulated, as when the blood had been—(1) exposed to formalin vapour for five minutes; or (2) treated with a mixture of ether and alcohol, equal parts of each; or (3) heated, as by passing the preparation five or six times through the Bunsen flame.

Wassermann and Schütze pointed out what the reader has doubtless already perceived—that *the* objection to the test is the extreme improbability of obtaining from any dried blood-stain a sufficient number of intact erythrocytes for the fact of their lysis having occurred to be easily appreciable. It is for this reason that the stain has found no favour with the medical jurists, for whose use it was devised.

Complement-Deviation Test.—Moreschi injected inactivated normal goat serum into rabbits, and obtained from them an antiserum which had a completely inhibitory effect

on the hæmolytic action on ox erythrocytes of inactive anti-ox-erythrocyte rabbit serum *plus* normal goat serum as complement. When normal rabbit serum was used as complement, the inhibition of hæmolysis was only slight, and when normal guinea-pig serum was used as complement, there was no inhibition of hæmolysis at all. He used as indicator a 10 perc ent. suspension of ox erythrocytes, and of this 1 c.c. was taken as standard. The antiserum was in quantities of 0·1 c.c., the complement being present in similar quantity in each tube, while the quantities of inactive hæmolytic serum varied.

The following table shows the results obtained :

Antiserum.	Complement.	Amboceptor Anti-ox-erythrocyte Rabbit Serum (56° C.).	Indicator.	Hæmolysis when Reactivation by—		
				Goat Complement.	Rabbit Complement.	Guinea-pig Complement.
0·1 c.c.	0·1 c.c.	0·001 c.c.	1 c.c.	0	0	Complete
		0·002 c.c.		0	0	,,
		0·004 c.c.		0	Complete	,,
		0·006 c.c.		0	—	,,
		0·008 c.c.		0	—	,,
		0·01 c.c.		0	—	,,
		0·02 c.c.		0	—	,,
		0·06 c.c.		0	—	,,
		0·08 c.c.		0	—	,,
One hour at room temperature						
	Two hours at 37° C.					

He then repeated the experiment, but used as the hæmolytic amboceptor an inactivated anti-ox-erythrocyte *goat* serum, and found that hæmolysis was completely inhibited in all the tubes : it made no difference whether the complement was derived from the fresh serum of the goat, the rabbit, or the guinea-pig.

In a third series of experiments he introduced into each tube 0·001 c.c. of inactivated normal goat serum, and found that with the anti-ox-erythrocyte *rabbit* amboceptor hæmolysis was inhibited in all cases.

The following table shows the results obtained:

Anti-serum.	Complement.	Normal Goat Serum (56° C.).	Amboceptor, Anti-ox-erythrocyte Rabbit Serum (56° C.).	Indicator.	Hæmolysis on Reactivation by—		
					Goat Complement.	Rabbit Complement.	Guinea-pig Complement.
0·1 c.c.	0·1 c.c.	0·001 c.c.	0·001 c.c. 0·002 c.c. 0·004 c.c. 0·006 c.c. 0·008 c.c. 0·01 c.c. 0·02 c.c. 0·04 c.c. 0·06 c.c. 0·08 c.c.	1 c.c.	No hæmolysis		
One hour at room temperature.							
Two hours at 37° C.							

He repeated this experiment, using as antiserum a serum obtained from a rabbit that had been immunized by injections of a solution of egg albumin, and instead of goat serum, he used a small quantity of egg albumin. The result was that hæmolysis was inhibited in every case. This he attributed to anticomplementary action.

Anti-serum, Anti-egg Albumin, Rabbit Serum.	Complement.	Egg Albumin.	Amboceptor.	Indicator.	Hæmolysis on Reactivation by—		
					Goat Complement.	Rabbit Complement.	Guinea-pig Complement.
0·1 c.c.	0·1 c.c.	0·000001 c.c.	0·001 c.c. 0·002 c.c. 0·004 c.c. 0·006 c.c. 0·008 c.c. 0·01 c.c. 0·02 c.c. 0·04 c.c. 0·06 c.c. 0·08 c.c.	1 c.c.	No hæmolysis		

The work of Moreschi stimulated Neisser and Sachs to carry out the following experiments:

1. 0·0015 c.c. of inactive anti-ox-erythrocyte rabbit serum, which was known to be hæmolytic for sheep's erythrocytes, was found to be enough to cause complete hæmolysis of 1 c.c. of a 5 per cent. suspension of sheep's erythrocytes in 0·85 per cent. salt solution, when the inactive antiserum was reactivated by the addition of 0·05 c.c. of fresh guinea-pig serum.

2. These quantities were found to cause complete hæmolysis when 0·1 c.c. of anti-human rabbit serum was also present.

3. The following results were obtained when, in addition, various normal sera were present in varying quantities:

	Normal Serum.	Hæmolysis with Normal Serum of—							
		Man.	Monkey.	Rat.	Pig.	Goat.	Rabbit.	Ox.	Horse.
	C.c.								
0·1 c.c. anti-human rabbit serum ...	0·01	0	0						
0·05 c.c. fresh guinea-pig serum as complement	0·001	0	0						
Normal serum	0 0001	0	Moderate	All complete hæmolysis					
1 hour at room temperature	0·00001	Trace	Complete						
Then add 0·0015 c.c. inactive hæmolytic serum as amboceptor and 1 c.c. of indicator	0·000001	Complete	Complete						
1-2 hours at 37° C. ...	0	Complete	Complete						

That is to say, when 0·0001 c.c. of human serum, or 0·001 c.c. of monkey serum, was present with 0·1 c.c. of anti-human serum, no hæmolysis occurred, while other normal sera had no inhibitory effect.

4. This experiment was repeated with three-months-old

blood-stains, caused by the blood of man, sheep, fowl, rabbit, guinea-pig, ox, and horse. These stains, which were on linen, were extracted with salt solution, and the extracts were diluted to various degrees, so high that in the higher dilutions no reaction could be obtained with the precipitin test. Yet in all cases results similar to those obtained with human and other sera were obtained by means of the complement-deviation test.

They recommended that the test be employed as a control of the results obtained by the precipitin test, on the ground that—(1) the absence or presence of hæmolysis is more easily appreciable than the beginning of a precipitin reaction; (2) a hæmolytic antiserum is easy to obtain—easier than a precipitating antiserum; (3) opalescence of the antiserum does not affect the test, as it does in the case of the precipitin test. They found that the rabbit is well suited for the purpose, and yields a fairly constant hæmolytic antiserum, whose minimum dose for 1·0 c.c. of a 5 per cent. suspension of sheep's erythrocytes lies between 0·15 and 0·25 c.c.

In another series of experiments they mixed the dilutions of stain-extract with the anti-human serum and 0·25 c.c. of normal rabbit serum, kept the mixture at 37° C. for an hour, and then added 1·0 c.c. of the indicator, and kept the tubes at 37° C. for two hours. The results obtained by using a fresh normal serum, which was of itself hæmolytic for the indicator, when known quantities of human serum were present, were as follows:

Quantity of Human Serum.		Hæmolysis.
C.c.		
0·001	+ Fresh hæmolytic serum,	0
0·0001	0·25 c.c.	0
0·00001		0
0·000001		Moderate.
0·0000001	+ inactive anti-human	Marked.
0·00000001	serum,	Complete.
0	0·01 c.c.	Complete.

That is to say, when so small a quantity of human serum as 0·00001 c.c. was present, 0·01 c.c. of anti-human serum

inhibited hæmolysis. They recommended that for forensic purposes an antiserum which will prevent hæmolysis, when 0·0001 c.c. of human serum is present, should be used. Friedberger worked with fresh guinea-pig serum, of which 0·5 c.c. had been ascertained to be the minimum dose required, 0·02 c.c. of an anti-human serum (generally from the rabbit), and 1·0 c.c. of various dilutions of homologous albumin (generally human serum), the tubes in all cases being filled to 1·5 c.c. with physiological salt solution, and kept at 37° C. for an hour, after which time there were added to the contents of each tube 1·0 c.c. of a 10 per cent. suspension of goat's erythrocytes that had been sensitized by contact with inactive anti-goat-erythrocyte rabbit serum, and washed thrice. As controls he used—(1) dilutions of heterologous sera, and (2) dilutions of homologous sera, with which only complement had been mixed. He found, using the anti-human serum obtained from a rabbit which Puppe had sent him, that 0·02 c.c. of this caused inhibition of hæmolysis if one part of human albumin in a thousand millions was present, although no precipitin reaction was obtained when one part in a thousand was present.

He expressed his opinion to the effect that the very delicacy of the test was a drawback, and that it was not absolutely reliable, as stains on a rag which were composed of human sweat and chicken's blood gave a 'human blood result.'

[The test is, however, one for human *albumin*, not for human blood only.—S.]

The results obtained by Ehrnrooth are given in the table on p. 85. He laid stress upon the need of ascertaining the minimum hæmolytic dose for 1 c.c. of a 5 per cent. suspension of the indicator erythrocytes, which must be well washed to remove from them all trace of serum, as otherwise there might be a precipitate formed, and this of itself might have the effect of inhibiting hæmolysis.

Muir and Martin found that many, but not all, complements are deviated by the combination of a substance with its antiserum. Anti-ox rabbit serum, when ox serum is present, causes deviation of complement in the case of guinea-

EHRNROOTH'S EXPERIMENTS.

Serum (55° C. for 30 Minutes).	Complement Fresh Normal Rabbit Serum.	Antiserum received from SCHULZ Precip. 1 : 40,000 in 30 mins.	Well-washed Goat Erythrocytes suspended in Salt Solution per Cent. (1 to 2 hours at 37° C.).	Amboceptor Inactive Anti-goat Erythrocyte Rabbit serum (1 to 2 hours at 37° C.).	Hæmolysis after 2 Hours at 37° C.
	C.c.	C.c.	Per Cent.	C.c.	
Human serum :					
1 : 1,000	0·25	0·01	5	0	0
1 : 10,000	0·25	0·01	5	0	0
1 : 20,000	0·05	0·02	10	0·00015	0
1 : 40,000	0·25	0·01	5	0	0
1 : 50,000	0·25	0·01	5	0	0
1 : 80,000	0·05	0·02	10	0·00015	0
1 : 100,000	0·25	0·01	5	0	H
8 per cent. salt solution	0·25	0·01	5	0	H
Inactive horse serum:					
1 : 1.000	0·25	0·01	5	0	H
1 : 50,000	0 05	0·02	10	0·00015	H

pig or rabbit serum; and anti-human rabbit serum in the presence of human serum does the same, the test being made by means of the hæmolysis of ox erythrocytes which have been sensitized by the amboceptor of guinea-pig or rabbit serum, or by means of rabbit erythrocytes similarly treated. Anti-ox rabbit serum *plus* ox serum also causes deviation of the complement of dog serum, as tested by the lysis of rabbit erythrocytes which is naturally caused by this serum; and anti-human rabbit serum *plus* human serum causes deviation of the complement of cat serum, as tested by its lysis of guinea-pig erythrocytes. But anti-ox rabbit serum *plus* ox serum causes no deviation of the complement of ox serum, tested by its lysis of rabbit erythrocytes; and anti-guinea-pig rabbit serum *plus* guinea-pig serum has no effect on the complement of rabbit serum, tested by its lysis of guinea-pig erythrocytes that have been sensitized by means of the amboceptor of rabbit serum.

They found that 0·05 c.c. of anti-human rabbit serum caused deviation of complement when 0·00001 c.c. of human

serum was present, although scarcely any precipitin reaction was obtained when 0·0001 c.c. was present, and a distinct reaction occurred only when as much as 0·001 c.c. was present. Of chimpanzee serum 0·0001 c.c. had the same effect as 0·00001 c.c. of human serum, and *Macacus rhesus* serum had almost the same action as chimpanzee serum. Their results were these:

Anti-human Rabbit Serum (55° C.).	Human Serum (55° C.).	Chimpanzee Serum (55° C.).	Complement of Rabbit Serum.	Deviated?
0·05 c.c.	0·00001 c.c.	0·0001 c.c.	0·05 c.c.	No hæmolysis

With the precipitin test their results were these:

Anti-human Serum (55° C.).	Quantity of Serum present.	Kind of Serum tested.		
		Human.	Chimpanzee.	Macacus.
0·05 c.c.	0·0001 c.c.	? Slight opalescence	0	0
,,	0·001 c.c.	Distinct precipitate	Slight, but distinct, precipitate	? Slight opalescence
,,	0·01 c.c.	Marked precipitate	Marked precipitate, but less than human serum gave	Slight, but distinct, precipitate

With anti-human rabbit serum no complement-deviation occurred in the presence of the serum of the ox, sheep, pig, dog, cat, mouse, guinea-pig, horse, or pigeon.

They agreed with Friedberger in holding that for forensic purposes an antiserum which causes deviation of complement in the presence of 0·00001 c.c. of its homologous serum should be employed.

Uhlenhuth reported that he had obtained complement-deviation with extracts of the following articles which he took from his collection: (1) Jute sacks; (2) military foot-bandages, new and old; (3) hemp-dust; (4) earth; (5) gravel; (6) bark;

(7) hay; (8) straw; (9) bread; (10) leather; (11) hair of various kinds; (12) woollen stockings; (13) linen; (14) trouserings of various kinds; (15) felt hats; (16) coats of various materials; (17) urine; (18) pepton; (19) pepton-bouillon; (20) various undiluted sera. He had failed to obtain the deviation with extracts of—(1) cotton-wool; (2) raw cotton; (3) dusters; (4) huckaback material; (5) a cambric bandage; (6) cork roofing; (7) pasteboard; (8) green leaves; (9) a linen shirt; (10) gauze; (11) twill; (12) beech-wood; (13) steel-shavings; (14) fireclay dust; (15) indiarubber tubing; (16) the lining of a waistcoat; (17) a pair of trousers; (18) rabbit fur. In Case 24 appended to Chapter V. (see p. 68) he had obtained complement-deviation, although the precipitin test was negative, and the blood on the sack was avian, as shown by the microscope. To this Neisser and Sachs replied that they had foreseen the occurrence of difficulties, and had recommended the use of two sets of tubes, the second set containing *boiled* extract of the stained material, as the specific complement-deviating power of the antigen is removed by boiling, while the power of non-specific substances to cause complement-deviation is unaffected by this process. In practice, as we shall see, the fallacies arising from the presence of complement-deviating substances other than antigen may be guarded against by using two series of tubes, in one the stain-extract being mixed with the antiserum, and in the other with salt solution or normal rabbit serum.

Schütze reported that he had found the test to be specific, and more delicate than the precipitin test. He recommended that it should have further trial by being employed alongside the precipitin test in practice.

Rickmann found that, while in the precipitin test, with the antisera at his command, the extent of specificity was only as 1 : 100, in the complement-deviation test it was as 1 : 10,000—in other words, the latter is 100 times more delicate than the former.

Certainly it is more delicate than the precipitin test, as I have more than once proved by experiment. The test is, however, not quite simple and easy to carry out. As we have

seen, a hæmolytic combination, of which the complement must always be fresh, and the amboceptor is of known hæmolytic power for the indicator, is required.

For the immunization of the animal from which we desire to obtain the hæmolytic antiserum we proceed thus: The animal whose erythrocytes are to be used is bled—conveniently by thrusting a trocar into the jugular—and the blood is received in a sterilized Erlenmeyer's or other flask, into which have been put sterilized steel-shavings. These are more convenient than glass-shot or porcelain pearls for defibrination of the blood, by its being shaken up with them. The defibrinated blood is decanted into centrifuge-tubes, the level attained by the blood in each being marked with a glass pencil, and the contents of the tubes are then centrifugated. The supernatant serum is then poured off, and stored for future use if required. The tubes are then filled up to the marked level with 0·85 per cent. salt solution, their contents shaken, and again centrifugated. The salt solution should be freshly made and sterile. The supernatant fluid having been poured off, the tubes are again filled up to the mark with salt solution, and their contents shaken and centrifugated. The supernatant fluid is poured off, and we have washed erythrocytes ready for use. These are then injected into the animal, enough salt solution being added to bring the mixture up to the original volume, of which the required dose is taken.* If the erythrocytes are required for the indicator, enough salt solution is added to them to make a 5 per cent. suspension. This will keep good for three or four days, if stored in a sterile flask and kept on ice.

Now, for the test we must determine the minimum quantity required of the

(1) *Hæmolytic antiserum;*
(2) *Complement;*
(3) *Anti-human serum;*

and this we proceed to do as follows:

* The dose may conveniently be 30 c.c., repeated after eight days, the rabbit being then bled eight to ten days after the second injection—intraperitoneal, of course.

I. The hæmolytic antiserum (anti-ox-erythrocyte rabbit serum) has already been rendered inactive, for purposes of storage, by being kept at 56° C. for half an hour. It must, therefore, be reactivated by the addition of complement, for which fresh guinea-pig serum is chosen. Of this a small quantity—0·1 c.c.—is added to each tube, in which are 1·0 c.c. of the indicator, and decreasing quantities of the hæmolytic amboceptor, beginning with 1·0 c.c. of a 1 : 10 dilution, the quantities where necessary having been made up to 1·0 c.c. by the addition of salt solution. The contents of the tubes are then well shaken and the tubes kept at 37° C. in the thermostat for two hours, and the result noted. As a control we have a tube in which there is no amboceptor.

The protocol of the determination is therefore this:

Tube.	Hæmolytic Amboceptor diluted (made up to 1·0 c.c. with Salt Solution where required).	Quantity of Amboceptor actually Present.	
1	1-10, 1·0 c.c.	0·1 c.c.	+1·0 c.c. ox erythrocyte 5 per cent. suspension indicator
2	0·5 c.c.	0·05 c.c.	
3	0·25 c.c.	0·025 c.c.	+0·1 c.c. fresh guinea-pig serum complement
4	0·15 c.c.	0·015 c.c.	
5	1-100, 1·0 c.c.	0·01 c.c.	
6	0·5 c.c.	0·005 c.c.	Two hours at 37° C.
7	0·25 c.c.	0·0025 c.c.	
8	0·15 c.c.	0·0015 c.c.	
9	0	0	Control of hæmolytic power of complement

As indicated above, in practice the quantities of salt solution required to make up the total volume of the dilution of amboceptor to 1·0 c.c. are first put into the tubes, then the required quantities of amboceptor-dilution, then the standard quantity of indicator, and lastly, the standard quantity of complement.

If, when the tubes are examined after they have stood in the thermostat for two hours, there be found considerable hæmolysis in the control-tube, it is clear that the guinea-pig serum of itself has a distinct hæmolytic action on the ox

erythrocytes in the dose used, and the determination must be repeated with a smaller dose of the guinea-pig serum —say, 0·05 c.c.—which, in order that the quantities in the tubes may be the same, will be 0·1 c.c. of a 1 : 2 dilution.

If, however, very little hæmolysis has occurred in tube 9, we fix the minimum hæmolytic dose of the amboceptor by observing the tubes in which hæmolysis is complete. The last of these, that in which is the smallest quantity of amboceptor, gives us the one limit, the first of those in which hæmolysis is incomplete giving us the other limit of the minimum dose. Thus, if hæmolysis be complete in tubes 1, 2, and 3, tube 3 gives us the highest limit; while tube 4, the first tube in which hæmolysis is incomplete, gives us the lowest limit, the minimum dose thus lying between 0·025 and 0·015 c.c.—*i.e.*, being 0·02 c.c. *for this particular rabbit.* For the test *twice this quantity* will be required—0·04 c.c., which equals 0·2 c.c. of a 1 : 5 dilution.

II. We have now to determine the minimum quantity of the complement which is required for the test. This determination must be carried out *every time* that the test is performed, for it is only fresh complement that can be used. Into each tube are put the required amounts of salt solution to bring the decreasing amounts of fresh guinea-pig serum up to 1·0 c.c.; in Series A the ascertained required amount of the hæmolytic amboceptor, in Series B only salt solution in the same quantity; and the standard amount of indicator; and the fresh guinea-pig serum, beginning with 0·1 c.c.—*i.e.*, 1·0 c.c. of a 1 : 10 dilution—and decreasing as far as may be judged convenient. The contents of the tubes, having been well shaken, are kept at 37° C. in the thermostat for two hours, and the result noted (see table on p. 91).

We then observe the tubes in which hæmolysis is complete and those in which it is incomplete, keeping note of those tubes of Series B in which hæmolysis has occurred as the result of the presence of complement alone, and deducting their number from the number of tubes in Series A in which hæmolysis has occurred. The required quantity of *this particular guinea-pig's serum is one and a half to two times*

Tube.	Fresh Guinea-pig Complement (made up to 1·0 c.c. with Salt Solution where required).	Quantity of Complement in Tube.	Series A.	Series B.	
	C.c.	C.c.			
1	1 : 10, 1·0	0·1			
2	0·75	0·075	0·2 c.c.	0·2 c.c.	+ 1·0 c.c. indicator
3	0·5	0·05	(ascertained	of salt	
4	0·35	0·035	required	solution	
5	0·25	0·025	quantity) of		
6	0·15	0·015	1 : 10 dilution		
7	0·1	0·01	of hæmolytic		
8	0	0	amboceptor		The tube contents well shaken. Tubes kept at 37° C. for 2 hours.

the minimum dose required for the reactivation of the ascertained required quantity of the hæmolytic amboceptor. Thus, if the minimum dose be between that in tubes 4 and 5—*i.e.*, between 0·035 and 0·025 c.c.—it is 0·02 c.c., and the required quantity will be between 0·045 and 0·06 c.c.—conveniently 0·25 c.c. of a 1 : 5 dilution of the serum.

III. For the determination of the required quantity of anti-human serum we take the ascertained required amount of complement, and mix this with decreasing amounts of the anti-human serum in two series of tubes. In the one series there is added 0·0001 c.c. of human serum, and in the other only salt solution to the contents of the tubes. The tubes are kept for one hour at 37° C., and then to their contents are added the standard amount of indicator and the ascertained required quantity of hæmolytic amboceptor, the whole being well shaken and kept at 37° C. for two hours, and the result noted. The results may also be noted again after the tubes have lain in the ice-chest overnight. We have then this protocol (see table on p. 92).

The object of having two sets of tubes is to eliminate any possible inhibitory action of the anti-human serum in excess: thus, if in Series A hæmolysis is inhibited in tubes 1, 2 and 3, while in Series B tube 1 also shows inhibition, it is evident that this quantity of antiserum—0·1 c.c.—is too

Tube.	Series A.	Series B.	Quantity of Dilute Anti-human Serum (filled up to 1·0 c.c. with Salt Solution where required).	Quantity of Anti-human Serum in Tube.	Fresh Guinea-pig Serum.
			C.c.	C.c.	
1	1 : 1,000 dilution human serum, 0·1 c.c. = 0·0001 c.c. of human serum	0·1 c.c. salt solution	1 : 10, 1·0	0·1	Ascertained required quantity
2	,,	,,	0·5	0·05	,,
3	,,	,,	0·25	0·025	,,
4	,,	,,	0·15	0·015	,,
5	,,	,,	1 : 100, 1·0	0·01	,,
6	,,	,,	0·5	0·005	,,
7	,,	,,	0·25	0·0025	,,
8	,,	,,	0·15	0·0015	,,
9	,,	,,	0·1	0·001	,,
10	,,	,,	0	0	,,
One hour at 37° C.					
+ 1·0 c.c. indicator. + ascertained required amount of hæmolytic amboceptor. Then two hours at 37° C.					

great, being of itself capable of causing inhibition. The minimum dose will lie between the amount in the last tube in which hæmolysis is incomplete and that in the first tube in which it is complete. If, then, we have hæmolysis in 1, 2 and 3 incomplete, and in 4 complete, the quantity lies between 0·025 and 0·015 c.c.—*i.e.*, is 0·02 c.c. For the test *twice this quantity* is required—that is, 0·2 c.c. of a 1 : 5 dilution.

As this anti-human serum is inactive, it will keep, and as long as the supply lasts the serum of *this particular rabbit* may be used in the above dose for the test.

When the test is carried out, we must first carry out the *precipitin test*, after having made the above determinations.

For the precipitin test, as well as for the complement-deviation test, in forensic cases it is well to have a series of control-tubes in which are known quantities of human serum.

We shall thus have absolute certainty of the efficacy of the antisera employed at the time of the tests.

Having carried out the precipitin test, the original stain-extract is taken and diluted to various degrees, and the complement-deviation test is carried out according to the following protocol:

Series A.	Series B.	Tube.	Stain-Extract Dilution.	Human Serum Dilution.	Quantity of Dilution in Tube (made up to 1·0 c.c. with Salt Solution where required).	
With anti-serum	With salt solution or normal rabbit serum instead of anti-serum	1	1 : 10	1 : 100	1·0 c.c.	+ascertained required quantity of anti-human serum inactive
,,	,,	2			0·5 c.c.	
,,	,,	3			0·25 c.c.	
,,	,,	4			0·15 c.c.	+ascertained required quantity of fresh guinea-pig serum as complement
,,	,,	5	1 : 100	1 : 1,000	1·0 c.c.	
,,	,,	6			0·5 c.c.	
,,	,,	7			0·25 c.c.	
,,	,,	8			0·15 c.c.	
,,	,,	9	1 : 1,000	1 : 10,000	1·0 c.c.	
,,	,,	10			0·5 c.c.	
—	—	11	0	0	1·0 c.c. salt solution	
One hour at 37° C.						
+1·0 c.c. indicator 5 per cent. suspension of ox erythrocytes. +ascertained required quantity of hæmolytic amboceptor. Two hours at 37° C.						

The results are noted and confirmed next morning, the tubes having stood overnight in the ice-chest: The tubes in Series B serve to eliminate the action of extraneous substances which may be present in the stained material. By using high dilutions of the stain-extract we obtain only the specific effect of the presence of homologous albumin. Sachs,

who had employed this method in twenty cases at the time, told me that he had not met with any vitiating influence which persisted in spite of high dilution.

As the ox, goat, and sheep are nearly allied as to their albuminous substances, if the precipitin test indicates the presence of the blood of one of these animals, the complement-deviation test should be carried out with a *guinea-pig erythrocyte* suspension as the indicator, the hæmolytic system being composed of fresh guinea-pig complement and inactive anti-guinea-pig-erythrocyte rabbit serum as amboceptor. Muir and Martin found, be it noted, that by immunizing rabbits with guinea-pig serum they obtained an antiserum which caused complement-deviation in the presence of its homologous albumin, although it did not cause the formation of a precipitate with it. It is also of interest to note that Friedberger, and also Liefmann,* found that, although anti-human serum that has been heated to 67° C. loses its power of causing the formation of a precipitate, it retains its power of causing complement-deviation in the presence of its homologous albumin.

* LIEFMANN, H.: 'Ueber die Komplementablenkung bei Präzipitationsvorgängen' (*Berliner klin. Woch*, 1906, 448).

CHAPTER VIII

SEROLOGICAL TESTS FOR BLOOD-STAINS: PRECIPITINS

In 1897 Kraus showed that by immunizing an animal by injections of a culture of a microbe, we obtain from the animal a serum which, when added to a filtered culture of this microbe, causes the formation of a precipitate in it. In 1899 Bordet showed that by immunizing a rabbit with intraperitoneal injections of milk which has been partially sterilized by being heated to 67° C., we obtain from the rabbit a serum which will cause the formation of a precipitate in this milk, which, when the test is carried out, should be filtered through filter-paper to deprive it of the excess of fat globules, since these, as they rise to the surface, would tend to carry the precipitate (casein) with them, and thus obscure the reaction, which may best be rendered evident by adding a few drops of the test milk to the antiserum mixed with normal rabbit serum. Wassermann and Schütze believed that lactosera are specific for the milks which have caused their production, since they found that human lactoserum reacted only with human milk, cow lactoserum with cow's milk, and goat lactoserum with goat's milk; but, as Nuttall has suggested, they may have been using *weak* lactosera, in which only the specific precipitins were demonstrable, for other observers have obtained results which differ from theirs.

Gengou reported that he had found that the reaction produced by a cow, goat, or sheep lactoserum on the milk of the cow, goat, or sheep is the same, but that a difference is apparent when human or mare's milk is tested.

Uhlenhuth reported that heating the tested milk to 114° C. for half an hour did not interfere with the reaction.

Many other substances have been found to give rise to the production of an antiserum which will cause the formation of a precipitate in their solution; but for our purpose all that is required is to mention the following facts:

In 1900 Myers reported that by injections of egg and serum albumin he had obtained specific antisera, which caused the formation of a precipitate—a 'precipitum,' as he preferred to call it—in solutions of these substances. Tchistovitch, by carefully dosing his animals with the exceedingly poisonous serum obtained from eels, was able to obtain a specific precipitating antiserum for eel serum. And Uhlenhuth, by injections of hen's egg albumin, obtained an antiserum which contained precipitins for solutions of the egg albumin of the hen, duck, goose, pigeon, and guinea-fowl; while by injections of goose's egg albumin he obtained an antiserum which caused marked precipitation in a solution of goose's or duck's egg albumin, and a less marked precipitation in a solution of hen's, pigeon's, or guinea-fowl's egg albumin.

Then Bordet showed that when a rabbit is immunized by injections of chicken's blood it yields a serum which causes agglutination and lysis of chicken's erythrocytes, and the formation of a precipitate in chicken's serum.

Stimulated by these observers' work, Nuttall carried out a large number of experiments with a view to ascertain the degree of specificity of precipitins. The experiments were carried out with the following antisera:

Antiserum for—	Number of Tests therewith.	Antiserum for—	Number of Tests therewith
1. Man	825	7. Hyæna	378
2. Chimpanzee ...	47	8. Dog	777
3. Ourang	81	9. Seal	358
4. Cercopithecus ...	733	10. Pig	818
5. Hedgehog ...	383	11. Llama	363
6. Cat	785	12. Mexican deer ...	749

Antiserum for—	Number of Tests therewith.	Antiserum for—	Number of Tests therewith.
13. Reindeer ...	69	22. Wallaby ...	691
14. Hog-deer ...	699	23. Fowl	792
15. Antelope ...	686	24. Ostrich	649
16. Ox	790	25. Fowl egg ...	789
17. Sheep	701	26. Emu egg ...	630
18. Horse	790	27. Turtle	666
19. Donkey... ...	94	28. Alligator ...	468
20. Zebra	94	29. Frog	551
21. Whale	94	30. Lobster ...	450

Grand total, 16,000 experiments.

These 16,000 tests were made with 900 specimens of blood, which represented at least 586 different species.

Friedenthal, in his work on blood relationship, writes somewhat as follows of the results of precipitin tests: It has thus become possible for biologists, who have all along accepted the Darwinian theory as being almost an axiom, to prove its truth to the non-biologists, who, although many of them were zoologists, botanists, and anthropologists, were inclined to the belief that what they termed the 'theory' of the descent of man was a mere hypothesis, partly unproved and partly erroneous, being in agreement with Driesch, the zoologist, who in 1896 wrote: 'Darwinism belongs to the history of our century, as does that other curiosity of the period—the Hegelian philosophy. Both are variations on the theme, "How a man may lead an entire generation by the nose," and are not calculated to elevate this century that is now drawing to its close in the opinion of future generations.' First we had the discovery by Dubois of the remains of *Pithecanthropus erectus*, the missing link between man and the extant anthropoid apes; then came Selenka's discovery that, like man, the anthropoid apes have a capsulated discoidal placenta, and thus differ from the other apes of the Old World; and now we have the discovery, by means of precipitating antisera, that the albuminous substances of the blood-serum of man are very closely related to those of the

blood-sera of the apes, the steps of the relationship from man backwards being definitely traceable. No one who knows Nuttall's work will demur to this opinion of Friedenthal. Nuttall showed that a 'mammalian reaction' occurs eventually in the sera of all mammals when an anti-mammalian precipitating serum is brought into contact with them, and that this general reactive power disappears on dilution, while the specific active power on the homologous serum is not thus removed, 'the reaction with the highest dilutions being practically specific.' The substance which causes antibodies to appear is conveniently designated *antigen*, and Obermayer and Pick showed that it is likely that besides the 'original' molecule groups in the albumin molecule of the antigen, which determine its specificity for the albumin of a species, there exist 'constitutive' groups, which determine its specificity for albumin as a whole. By introducing iodo-, nitro-, or diazo-groups into the albumin molecule, they succeeded in removing the specificity for a species; for precipitins obtained by immunization with such modified albuminous bodies reacted with corresponding substances formed by treatment of the albumin of no matter what species of animal. Iodine albumin gave rise to precipitins which reacted with all iodine albumins, etc. The action is chemical, as shown by Myers, Michaelis, and others; and quantitative, as Eisenberg and also Strube have shown. The precipitins are contained in the globulins of the antiserum, of which Corin considers the paraglobulin to be of most importance, a fact which, as we shall see, he utilized in the method of preparation of antisera devised by him. So far as I am aware—and I have read more extensively than the bibliography would indicate—no one save Whitney has found that any difference in specific gravity, alkalinity, or any other physical property, exists between an antiserum and the normal serum of the same animal.

Leclainche and Vallée have shown that the precipitate consists of albumin, which, according to Hopkins (as reported by Nuttall), contains a large amount of phosphorus in organic combination. From the experiments carried out by

Graham-Smith and Sanger with a view to settle whether the amount of salt in the tested serum really influences precipitation, it appears that the greater the amount of salt the more flocculent the precipitate, and the longer it takes to settle, owing to the greater density of the medium. At first we have the formation of numberless microscopic particles, which causes a slight cloudiness at the point of contact of antiserum and homologous solution, which cloudiness tends to spread throughout the solution. Then the particles coalesce, forming larger particles, and finally flakelets, which may be seen with a magnifying glass, and which grow larger, so that in the end they are to be seen with the naked eye. The flakelets are deposited at the bottom of the test-tube and on its sides, the supernatant fluid being clear, although it may still contain some precipitable substance—precipitogen —for whose precipitation there are no precipitins available, all having been already used up.

Naturally, when the specificity of the precipitins was being established, observers endeavoured to ascertain whether they could differentiate various forms of albumin by them. Thus Leclainche and Vallée thought that they could, by testing urine with the appropriate antisera, distinguish the various forms of albumins present in it; but Linossier and Lemoine in their work on this subject were unable to confirm this observation, and Halban and Landsteiner found that the antiserum obtained by treating rabbits with bull semen, caused a precipitate to form in ox blood-serum; while Strube found that immunization by means of human spermia and human testicular extract give rise to an antiserum whose action on solutions of human semen was in no respect different from that obtained with an antiserum obtained by immunization with human serum. Again, Graham-Smith and Sanger found that the anti-human serum which they used caused the formation of a precipitate in old or fresh human serum, placental serum, pleuritic exudation, hydrocele fluid, ovarian cyst fluid, and amniotic fluid. The action, then, is a *general albumin reaction.*

Kraus had observed that 37° C. is the optimum tempera-

ture for the precipitin reaction, and this has been confirmed by the work of Myers, of Wassermann and Schütze, and of Strangeways, as reported by Nuttall, who states that he considers that faulty observations were the cause of Kister and Wolff believing that the temperature makes no difference.

Dieudonné injected albuminous urine, pleuritic exudation, and blood-serum of man into rabbits, 10 c.c. being the dose, which was repeated every third or fourth day, and obtained anti-human sera, which he tested by mixing them with solutions of the blood of the rabbit, guinea-pig, goose, and pigeon, and on solutions of human serum, albuminous urine, and pleuritic and ascitic exudations. He found that only human albuminous fluids were affected, and that normal rabbit serum had of itself no precipitating power, such as is possessed by the serum of an immunized rabbit.

Schirokich is reported—I cannot read Russian—to have immunized rabbits with human placental blood, and to have thus obtained an anti-serum which reacted with extracts of two-year-old human blood-stains; while no reaction occurred with the bloods of the cat, ox, goat, pig, horse, camel, rabbit, and guinea-pig.

Schütze obtained the reaction with a solution of seminal stains that were six months old.

Whittier found that his anti-human serum reacted with human blood, but not with the blood of the horse, ox, rabbit, or guinea-pig, and Butza found that his anti-human serum reacted only with human blood, and not with the blood of the dog, cat, pig, ox, sheep, rabbit, guinea-pig, fowl, pigeon, turkey, duck, goose, or fish.

Biondi obtained a precipitate in solutions of human milk and saliva, but not in solutions of the milk or saliva of the cow, goat, or ass. He noted that it did not matter whether the person whose blood was tested was healthy or diseased, and that putrefaction had no effect on the reaction. He failed to obtain an anti-human serum by immunizing a monkey.

Ewing found that high dilutions of the blood of four kinds

of monkey failed to give the reaction, while equivalent dilutions of human blood still continued to give it.

Ziemke obtained from Wassermann and Schütze two rabbits, A and B, of which A had received the greater number of immunizing injections, and with the antisera obtained from these rabbits he tested the following articles:

1. **Fresh Blood** of man, cat, horse, dog, ox, sheep, pig, mouse, rabbit, guinea-pig, calf, and lamb. Antisera A and B caused the formation of an opacity within fifteen minutes in the case of human blood only, the opacity having cleared up and a flocculent precipitate having been thrown down after twenty-four hours.

2. **Dried Blood** of man, two years old, of ox, twenty-five, thirty-two, and thirty-six years old. A and B caused an opacity within three hours in the case of human blood only, and this remained unchanged up to twenty-four hours.

3. **Blood-stains** of human blood on shirting twenty-eight and thirty-three years, on gauze two, and on linen seven years old; on linen eight weeks old of the blood of the sheep, calf, pig, dog, ox, horse, and rabbit. The solutions were made in each case—(1) with 1 per mille soda solution, and (2) with 0·75 per cent. salt solution; and the soda solutions were tested with antiserum A at room temperature, the salt solutions being tested with antiserum B at 37° C. in the thermostat. A gave an opacity with human blood solutions only (the twenty-eight-years-old stain did not dissolve), and the opacity remained unchanged on the following day. B showed a slight opacity with rabbit blood only.

4. **Blood in Earth.**—Human blood that had lain in garden mould one and three years; bloods, for eight weeks mixed with earth, of the horse, ox, sheep, calf, and pig. A gave an opacity only with human blood, the opacity remaining unchanged for twenty-four hours; B gave an opacity only in the case of the one-year-old blood.

5. **Blood from a Case of Carbon-Monoxide Poisoning,** that had lain on linen, or been mixed with garden mould for eight weeks. A gave a moderate opacity after one hour,

which remained unchanged after twenty-four hours. B gave no opacity.

6. **Blood-stains Five Years Old on a Rusty Knife-blade and on a Bright Axe-blade.**—A gave a moderate opacity within one hour, and this was unchanged after twenty-four hours. With a solution which contained rust alone no reaction was obtained.

7. **Washed-out Blood-stains.**—These had been caused by human blood, and were still visible as faint yellowish stains on a linen cloth, on which there were also rust-spots. A gave a slight opacity in the soda extract in one hour. No reaction was obtained with the extract of one of the rust-spots.

8. **Human Blood on Plaster.**—The stains were two years old. A and B caused a marked opacity to appear after three hours.

9. **Human Blood-stains on Wood.**—With stains three years old on matches A gave a moderate opacity after three hours; with stains one year old on a branch B gave a slight opacity after three hours.

10. **Human Blood-stains on Glass.**—A gave an intense opacity after one hour in the case of four lentil-sized blood-drops on a window-pane three months old.

11. **Human Blood-stains on Linen exposed to Weather for Seven Months.**—A gave a marked opacity after three hours.

12. **Human Blood-stains on Paper Ten Years Old.**—A gave a slight opacity after three hours.

13. **Blood from a Three-Days-old Corpse.**—B gave an intense opacity in a few minutes, which after twenty-four hours had partly cleared up, leaving a flocculent precipitate.

14. **Putrefied Bloods** from a case of carbon-monoxide poisoning, and of the pig, ox, goose, pigeon, and fowl. B gave an intense opacity with human blood, and no reaction with other bloods.

He reported that he had also obtained satisfactory results by making an extract of the stain with a concentrated solution of cyanide of potassium, and adding to this tartaric acid until the extract had become almost neutral, then decanting

MATERIAL FROM SCOTLAND YARD EXAMINED BY *GRAHAM-SMITH* AND *SANGER*, WITH THE RESULTS OBTAINED BY THE PRECIPITIN TEST (FROM NUTTALL)

Material.	Age.	Foam Test.	Character of Solution.	Reaction to Litmus.	First Series.		Second Series.	
					Anti-human I.	Normal Rabbit.	Anti-human II.	Anti-ox.
1. Lining of clothes	3 years	Good	Clear	Neutral	Marked cloud, 60 mins.	Nil	—	
2. Felt hat	10 ,,	,,	Cloudy, clear after filtering	,,	—	—	Cloud, 15 mins.	Nil
3. Printed paper	11 ,,	,,	Clear	Alkaline	Cloud, 5 mins.	Nil	Cloud, 60 mins.	,,
4. Part of same paper	11 ,,	,,	,,	Neutral	—	—	Cloud, 15 mins.	,,
5. Same scorched*	11 ,,	,,	,,	,,	Nil	,,	Nil.	,,
6. Alpaca dress	11 ,,	,,	,,	,,	Immediate cloud	,,	Marked cloud, 10 mins.	,,
7. Braid†	11 ,,	,,	,,	Alkaline†	Slight cloud, 60 mins.	? Cloud	Slight cloud, 60 mins.	,,
8. Cardigan jacket	11 ,,	Fair	Cloudy, clear after filtering	,,	—	—	Marked cloud, 30 mins.	,,
9. 'Black rep'	11 ,,	Slight	Clear	Slightly acid‡	Slight cloud, 60 mins.	Nil	Slight cloud, 15 mins.	,,
10. Cotton fabric,† apparently washed	11 ,,	Fair	,,	Alkaline†	Slight cloud, 5 mins., no increase	,,	—	
11. Hair	28 ,,	Good	,,	Neutral	Cloud, 30 mins.	,,	Marked cloud, 5 mins.	,,
12. Wooden handle of chopper§	28 ,,	None	,,	,,	—		Nil	,,

* Capacity for reaction probably destroyed by heat.
† Alkaline solution probably retarded development of reaction.
‡ The acidity may have interfered with the reaction.
§ The cause of the absence of reaction unknown.

Only a small quantity of blood present on these articles, save No. 11.

and filtering the extract, and diluting the filtrate till it was of a pale yellowish-red colour. He found that if the extract be acid it will never become clear, no matter how often it be filtered, whereas if it be but slightly alkaline it remains clear, and fit for testing. I have found that with a cyanide extract prepared in this manner one obtains fair results with the test.

Graham-Smith and Sanger examined several articles which had been placed at their disposal by Mr. Henry of Scotland Yard, with the results noted in this table, which I take from Nuttall's book (see table on p. 103). As we shall see, the forensic test is more one of *time-reaction.*

The following table, which I take from Nuttall's book, shows the effects of heat on the power of a precipitating antiserum:

Kind of Antiserum.	Temperature C. to which it was exposed.		Remarks.	Authority.
	Destroyed at—	Resisted.		
Hæmatoserum	70°	—	—	Tchistovitch, 1899
,,	70°	65°	Though weakened in power	Bordet, 1899
,,	—	60°	No effect apparent	Rostoski, 1902
,,	—	60°	½ hour, still effective	Obermayer and Pick, 1902
,,	65° in 24 hours	60°	48 hours, weakened	Linossier and Lemoine, 1902
,,	72°	—	—	Eisenberg, 1902
,,	68°, 2 hours (almost quite destroyed)	52°	No effect	Michaelis, 1902
Anti-egg albumin	—	60°	1 hour, scarcely affected	Uhlenhuth, 1900
Anti-urine (rabbit treated with human albuminous urine)	—	58°	2 hours, still effective	Leclainche and Vallée, 1901
Lactoserum ...	—	56°	'Resisted'	Moro, 1901
Bacterioserum	58°	—	—	Kraus and v. Pirquet, 1902
,,	58°-60°	—	Destroyed in ½ to ¾ hour	Pick, 1902

The next table, also from Nuttall, shows the effect of heat on the precipitogen; but these results, as we shall see, do not apply to blood-stains.

Substance heated.	Destroyed at—	Resisted.	Remarks.	Authority.
Eel serum ...	80°	58°	But gave less reaction	Tchistovitch, 1899
Fowl egg-white	—	56°	½ hour, not appreciably affected	Myers, 1900
Ox and sheep serum globulin solutions	—	56°	,,	,,
Fowl serum ...	—	70°	½ hour	Bordet, 1899
Human albuminous urine	—	58°	2 hours	Leclainche and Vallée, 1901
Milk	100°, ½ hour	—	No reaction	Schütze
,, ...	—	100°	½ hour, still reacted	Müller
Egg-white solution	78°, 1-1½ hours	—	No reaction	Eisenberg, 1902
Serum dilution (1 : 100)	100°, 5 mins.	55°	½ hour, no effect	Nuttall, 1901
Serum dilution 1 : 10	65°, 24 hours	60°	4 days, no effect	Linossier and Lemoine, 1902

From the experiments carried out by Biondi, Graham-Smith and Sanger, and Ponzio, it appears that blood-stains resist interference very successfully.

Graham-Smith and Sanger experimented with blood-stains that had been found on the following articles, and found that the precipitin reaction was not affected. In all cases I have given the English alphabetical order, for convenience' sake. Canvas, brown; cloth of various kinds—dress material, coarse duster, merve, serge, ticking, tweed, velveteen; coal; coins—copper and silver; cork; felt (hat); flannel; flint; leather of various kinds—'chamois,' 'suède,' white kid, boot—patent and yellow (thick polished yellow leather had an inhibitory effect); linoleum; paper of sorts; paper (wall) of various kinds—blue, brown, green, red, and yellow; rubber; silk; slate; stones; string; straw. In many cases they found that the extract of the stain was *acid*, or *too alkaline*, and required to be neutralized before the reaction was obtained.

Biondi found that the following substances had no effect on the blood-stains that had been deposited on them: bone; brass; brick; clay; copper; earthenware; glass; horn; iron, even when rusty; leather, plain or coloured—in the case of coloured leather the solution was turbid, and required filtration; linen, even if the stained cloth had been exposed

to rain, dew, and sunlight for a long time; mortar; paper—white or coloured, written or printed; porcelain; sand; silver; stone; vegetables—fresh or dried; wood—rough, polished, or varnished.

With a view to ascertain the effects of various poisons on the precipitable substance in the blood, Ponzio poisoned dogs with the following substances, but could find no difference between the reaction of their blood and that of the blood of normal animals: Abrin; acids; alcohol; alkalis; antipyrin; coal-gas; curare; male-fern oil; lead; mercuric chloride; morphin; nitro-benzin; phosphorus; potassium bromide, chlorate, cyanide, iodide; ricin; salicylic acid; strychnine.

Graham-Smith and Sanger, however, state that if blood be *intimately* mixed with lime it becomes completely destroyed. Biondi's observation was probably made with hard, well-dried mortar, such as one finds on a wall, especially in a sunny land.

The observations of Nuttall, Uhlenhuth, Biondi, and others have shown that putrefaction does not affect the reactive power of blood.

Biondi, too, found that blood-stains produced by the crushing of fleas, bugs, and mosquitoes, gave the reaction, which is a point of great importance in the tropics, where these and other blood-sucking insects abound.

We have now to consider the conditions which are adverse to the reaction. Graham-Smith and Sanger found that the reaction is inhibited by the presence of: acids; alkalis; cupric sulphate, 1 part in 100,000; formalin, 1 part in 100; lysoform; lysol; mercuric chloride, 1 part in 10,000; silver nitrate; and thymol. Vincent found that the reaction is affected by these acids: acetic, citric, lactic, oxalic, and tartaric, if present 5 parts in 1,000; and by these alkalis: ammonia, caustic potash, and caustic soda. But if the extract of the stain be neutralized, some reaction will be obtained in these cases. On the other hand, the interference caused by the following substances cannot be removed: Calcic chloride;

cupric sulphate; ferric sulphate; mercuric chloride; potassium permanganate; sodium bisulphide; and zinc chloride.

Ferrai observed—and the observation was confirmed by Biondi—that no reaction can be obtained from blood that has been exposed to a temperature of 130° C. for an hour, 140° C. for twenty minutes, 150° C. for ten minutes, or 160° C. for from five to ten minutes. And Mirto found that bloods that have been exposed to temperatures between 100° and 120° C. are soluble in physiological salt solution, and the extract gives the reaction; but that temperatures between 130° and 140° C., if the exposure have lasted half an hour, greatly reduce their solubility, and if the exposure have lasted an hour, entirely destroy it, and consequently no reaction can be obtained.

It is of interest, too, to note that di Mattei found that the presence of sulphuric acid or bisulphide of carbon interferes with the reaction, and that even so small a quantity of potassium permanganate as 1 part in 1,000,000 adversely influences it.

CHAPTER IX

SEROLOGICAL TESTS FOR BLOOD-STAINS: PRECIPITINS *(Continued)*

THE idea that the specificity of the precipitin reaction might be turned to account in forensic medical practice occurred at the same time to Uhlenhuth and to Wassermann and Schütze, and from the experiments made by these observers, which have been repeatedly confirmed by others, has been derived the test which is now accepted in the Courts of most civilized countries as a most valuable addition to the means at the command of the medico-legal expert for obtaining evidence as to the source of a blood-stain.

Wassermann and Schütze had tried, by injections of defibrinated human blood into rabbits, to obtain an antiserum which would cause agglutination and hæmolysis of human erythrocytes, and thus be of service for diagnosis; but they abandoned this attempt on finding that the amount of test-blood required was relatively large, and that this must be fairly fresh, so that well-preserved erythrocytes might be still present in it. They then tried by injecting erythrocyte-free serum to obtain specific precipitins for human serum, such as they had already obtained for milk, on the supposition that as the precipitins act, not on the formed elements of the blood, but on the globulins of its serum, the blood to be tested might be older than that necessary for the test by agglutinins and hæmolysins—as the presence in it of formed elements was not necessary.

They injected 10 c.c. of erythrocyte-free human serum into rabbits every second day or so for five or six injections, the

animals being bled on the sixth day after the last injection, and the blood kept on ice till the serum had separated from the clot. When 0·5 c.c. of this serum was added to human serum diluted with physiological salt solution, or to a lake-coloured solution of blood in distilled water, an abundant cloudy precipitate was formed at room temperature, and more markedly when the tubes were kept at 37° C. in the thermostat.

Lake-coloured solutions of the blood of man, monkey, cat, dog, pig, ox, sheep, goat, horse, ass, rabbit, guinea-pig, rat, mouse, fowl, duck, goose, sparrow, eel, pike, and tench, were tested with this anti-human serum, and they found that a precipitate was formed only in the human and simian blood-solutions, that in the latter being the less in amount and the slower of appearance. This property of monkey blood, they considered, might be left out of count as far as Germany was concerned, from the forensic point of view. They then tested blood-stains three months old, caused by various bloods on linen, knives, and various instruments. The stains had become brownish, owing to the formation of met-hæmoglobin. They soaked a stain of the size of a ten-pfennig piece [about the size of a sixpence—S.] in 5 to 6 c.c. of physiological salt solution, and obtained a dirty brown turbid liquid, which on being filtered through filter-paper became clear. On the need of having a clear liquid, so that the results obtained may be sure, they insisted strongly, and remarked that stains made by the blood of birds and fishes require their extracts to be repeatedly filtered, so that the fatty particles and masses of nuclei may be removed.

Of the clear extracts 4 to 5 c.c. were put into test-tubes, and into each test-tube was then put 0·5 c.c. of the anti-human rabbit serum. The tubes were then kept at 37° C., and the reaction noted. After twenty minutes the human blood-stain extract had become distinctly turbid, the simian extract showed the beginning of a slight turbidity, while the other extracts remained clear; after thirty-five minutes the human extract showed a distinct flaky precipitate. As controls they employed (1) extracts to which had been

added normal rabbit serum, and (2) a tube in which there was anti-human rabbit serum alone. All these showed no change.

Uhlenhuth tested his method of determining the source of a blood by means of a specific precipitating antiserum on the putrefied bloods of man, horse, ass, ox, sheep, dog, cat, goose, fowl, hare, rabbit, and stag. The human blood-solutions showed a turbidity, while all the others remained clear. The solutions were obtained by diluting the bloods with a large quantity of physiological salt solution, and filtering the solutions through a sterile Berkefeld filter, by which means a perfectly clear sterile liquid was obtained, of which 4 c.c. were taken, and to this quantity 12 drops of the antiserum were added. It is worthy of note that in some cases the human blood tested had undergone putrefaction for three months.

He then tested the urine of a menstruous woman, and specimens of urine with which there had been mixed blood from the ox, pig, sheep, horse, cat, and fowl. The menstrual urine—in which, of course, some blood was present—alone reacted.

Then he experimented with water that had been used to wash bloods with the aid of alkaline soap. The washings of human blood alone reacted.

As these experiments seemed to point to the sure specificity of the reaction, Uhlenhuth then set to work with blood-stains of—to him—unknown origin, and correctly diagnosed human blood in the following cases: (1) Blood-stains on a bludgeon; (2) blood mixed with sand, five years old; (3) blood-stains on a woollen cloth, four years old; (4) blood on the fork and slit of a pair of trousers, from a case which had at first been suspected to be one of rape, but was found to be one of coitus with a menstruous woman; (5) blood on an axe-haft, one year old. Further, he correctly diagnosed the source of the blood in the following cases: (1) Pig's blood on a cloth, many years old; (2) dried pig's blood, four years old; (3) dried human blood, one year old; and (4) a mixture of sheep's and pig's blood, twelve years old.

On further experiment he found that an anti-pig rabbit serum gave a precipitate with pig's blood, and also, in a less degree, with wild pig's blood, and that an anti-horse rabbit serum gave a precipitate with horse's blood, and also, in a less degree, with the blood of an ass. Also that anti-fox serum precipitated fox blood, and also, to a less extent, dog's blood, while anti-cat serum precipitated cat's blood alone, there being no blood of another of the *Felidæ* at his disposal. Most notable of all, with anti-ox serum he observed that a precipitate formed in ox blood, sheep's blood, and goat's blood.

In all cases the bloods of other animals were unaffected by the antisera noted, the experiments being carried out in each case with the bloods of man, ox, horse, ass, sheep, goat, pig, bat, fowl, pigeon, goose, duck, owl, crow, sparrow, rabbit, guinea-pig, rat, mouse, leech, dog, fox, cat, and stag.

Stern found that while anti-human rabbit serum caused a precipitate to form in solutions of dried human blood and albuminous human urine, and slightly in solutions of the blood of three kinds of monkey, no reaction was observed in the case of the blood of the horse, ox, sheep, or pig.

From all that has gone before, it is abundantly evident that, while the reactive power of human blood-stains to the precipitin test is remarkably stable, it is important to note whether the extract which we have obtained is acid or alkaline, and to take steps to neutralize it if it be acid, and to reduce its alkalinity if it be alkaline.

The reader, too, will have noted the fact that the similarity between human and simian albumin is great enough to be a source of possible error. With a view to prevent the occurrence of mistakes, which might easily lead to grave results, various methods of procedure have been proposed.

We have already seen that high dilution of the tested serum of itself tends to extinguish the reactive power of simian albumin; but obviously it is difficult to say that we have carried out dilution beyond the simian reaction-point when we are dealing with an extract of a dried blood-stain.

Weichardt made several experiments, which may be briefly described as follows:

1. To the antiserum obtained from a rabbit immunized by injections of blood from horse I., he added some serum from horses II., III., or IV., and centrifugated the mixture, which he found now caused the reaction with the serum of horse I. only.

2. He immunized a rabbit with four doses of 5 c.c. of serum of woman I. mixed with a little phenol, and on the twenty-sixth day he obtained 40 c.c. of blood, of which the serum was such that 0·3 c.c. of it, when added to 0·1 c.c. of human blood that had been made up to 10 c.c. with salt solution, caused a precipitate to form immediately, this human serum having had 1 part in 200 of phenol added to it, and having been kept in the ice-chest for a month.

To some of this antiserum he added one-tenth of its volume of monkey serum, left the mixture in the ice-chest for fifteen hours, and filtered it through a clay filter; to the filtrate he added one-tenth of its volume of monkey serum, and again filtered the mixture after it had stood for fifteen hours in the ice-chest. Of this simianized antiserum he added 0·5 c.c. to 0·1 c.c. of human blood, made up to 10 c.c. with salt solution, and after fifteen minutes this became turbid, giving a woolly precipitate after five hours; 0·5 c.c. added to 0·1 c.c. monkey serum A, made up to 10 c.c., gave no reaction for nine hours, after which time a fine precipitate was formed, and the same result was obtained with a similar dilution of monkey serum B.

3. This anti-human serum was treated with serum from woman II. in a similar manner, and of the so-treated serum 0·3 c.c. was added to 0·1 c.c. of serum of woman I., made up to 10 c.c. with salt solution, with the result that a turbidity at once appeared, and a precipitate formed after thirty minutes. On the other hand, with a similar dilution of the serum of woman II. there was only a slight turbidity evident after nine hours.

4. A rabbit was immunized with blood from corpse I, and the resulting antiserum was twice treated with one-tenth of its volume of blood from corpse B, the mixture being each

time left for fifteen hours in the ice-chest and then filtered. The resulting serum gave a marked precipitate with solutions of blood A, but only the beginning of a precipitate with blood B. These conclusions prompted Ehrnrooth to carry out a series of experiments, as the result of which he reported that in three cases in which he had used powerful antisera he failed to distinguish between the blood of different individuals, a positive result being obtained only when weak antisera were used. It is worthy of note that Weichardt said that in the experiment D he found that a rabbit which had received 1 c.c. of blood, mixed with 0·5 per cent. of its volume of phenol, every day for five days, on the eighth day yielded a good antiserum.

Uhlenhuth had to obtain an antiserum for hare's blood, and, as the rabbit is closely akin to the hare, he thought that it was not likely that by injections of hare's blood he could obtain an anti-hare rabbit serum. So he also immunized some fowls with a solution of old dried hare's blood in physiological salt solution, and obtained an antiserum which reacted with rabbit's blood as well as with hare's blood. The anti-hare rabbit serum, on the other hand, caused a precipitate to form only in a hare's blood solution. Repeating these experiments with pigeon's blood injected into fowls, he found that the anti-pigeon fowl serum gave a precipitate in twenty minutes with a 1 : 4,000 dilution of pigeon's blood, but no precipitate with a fowl's blood-solution. Encouraged by these results, he immunized two *Cercopitheci fuliginosi* and one *Macacus rhesus* with human serum, by subcutaneous injections of 5, 10, 15, 20, and 30 c.c. of fresh serum. One of the *Cercopitheci* and the *Macacus* yielded good antisera, which formed a precipitate in a solution of human blood, but none in a solution of monkey's blood. This method of immunization against the serum of a closely related species he called 'cross-immunization.' Pursuing his experiments further, he obtained from two specimens of *Macacus rhesus* and three of *Cercopithecus collaris* antisera which reacted with human serum diluted 1 : 1,600 and 1 : 2,000 respectively.

With this anti-human monkey serum he carried out a series of complement-deviation tests with human blood and with the blood of the following monkeys: (1) Chimpanzee; (2) gibbon; (3) orang-outang; (4) mandrill; (5) baboon; (6) *Cercopitheci mona, collaris, Werneri, pygerythrus;* (7) *Macaci rhesus, cynomolgus;* (8) *Cynocephali sphinx, babuini;* (9) *Cynocephalus niger;* (10) *Cebus capucinus;* (11) *Ateles Geoffroi.* He found that, while anti-human rabbit serum caused complement-deviation in the presence of human and of monkey blood, anti-human monkey serum caused it only when human blood was present.

He also noted that rabbits immunized with the blood of wild rabbits did not yield an antiserum which caused precipitation or complement-deviation, and that he had not found iso-precipitation to occur, though this had been reported by Schütze.

Hamburger endeavoured to distinguish easily between the blood of the ox, goat, and sheep by means of the precipitin test, since, as a rule, these bloods all react to the antiserum produced by immunization with one of them. He found that with anti-goat rabbit serum the precipitate formed in goat's serum is more marked than that formed in ox or sheep's serum; with anti-ox rabbit serum the precipitate formed in ox serum is more marked than that formed in goat's or sheep's serum; and with anti-sheep rabbit serum the precipitate formed in sheep's serum is more marked than that formed in ox or goat's serum. He recommended that when the extract of a stain gives a precipitate on treatment with anti-ox, anti-goat, or anti-sheep serum, it should be treated with all three antisera, and the most marked precipitate will be that caused by the antiserum for the blood of the animal—ox, goat, or sheep—from which the blood that caused the stain was derived. In his experiments he read off his results five minutes after mixing the antiserum with a 1 : 100 dilution of the normal serum that was being tested, the dilution being made with physiological salt solution.

Bearing on this point we have the proposal made by

Dehne to control the results of the test by heating the tubes in which a turbidity has been produced with the normal serum of the animal against which the antiserum has been made. He found that by thus using the antigen the tested material was rendered quite clear, while if only a mammalian reaction were the cause of the turbidity, this had after twenty-four hours developed into a precipitate. Having obtained a turbidity in a solution of a stain with anti-human serum, he treated portions of this solution with normal sera—horse, rabbit, human—and with physiological salt solution. Thus:

To Part of Clouded Solution.	Result after 10 Minutes.	Result after 24 Hours.
1. Horse serum	Turbid	Precipitate
2. Rabbit serum	,,	,,
3. Human serum	Clear	Clear
4. Salt solution	Turbid	Precipitate

He considered that this solution of the precipitate in excess of antigen formed a valuable corroborative test, as it is the result of specific solubility. ['Die spezifische Löslichkeit und ihre Anwendung bei der forensischen Blutuntersuchung.' *Münchener med. Woch.*, 1207, 357.] I do not think that this elaboration of the test is required.

CHAPTER X

SEROLOGICAL TESTS FOR BLOOD-STAINS: PRECIPITINS (*Continued*)

We have now to consider the method of carrying out the test in forensic practice. The suspected stain, which has been proved to be due to mammalian blood, should be extracted with 0·85 per cent. salt solution if possible. Stains of which an extract cannot be obtained with salt solution, may be treated with a solution of potassium cyanide, and the extract thus obtained nearly neutralized by carefully adding to it a solution of tartaric acid, the extract being then diluted with salt solution. The presence of serum in the extract may, as first pointed out by Nuttall, be ascertained by gently blowing through a pipette into it. If serum be present, there will be formed bubbles, which tend to persist. This 'foam test' is more reliable than the colour test, on which Uhlenhuth at first relied, and Ziemke with him. We should also have a 1 : 1,000 dilution of human serum, as a control of the potency of our antiserum, and extracts of various blood-stains, as a control of its specificity. Uhlenhuth keeps a stock of blood-stains of various ages, caused by the bloods of various animals, so that he may always have at hand a stain of the kind alleged to be present in that under examination, and of nearly the same age.

Our antiserum must be one which, when 1 part of it is added to 20 parts of a 1 : 1,000 dilution of homologous blood, causes at the point of contact a cloudiness to appear at once, or at latest within one to two minutes. When a 1 : 10,000 dilution is used within three minutes, and when

a 1 : 20,000 dilution is used within five minutes, the beginning of a cloudiness should be apparent, according to Uhlenhuth and Beumer. For practical purposes a 1 : 10,000 dilution is as high as one need go. Into each of the taper test-tubes are pipetted 2 c.c. of the extracts, and their dilutions—the greater the dilution the more specific the reaction—and then 0·1 c.c. of the antiserum is allowed slowly to run down the side of each tube, and the reaction is observed every five minutes up to twenty minutes. In the control-tubes are—(1) as noted, a 1 : 1,000 solution of human blood; (2) an extract of a stain of like kind and age; (3) extracts of various other blood-stains; (4) the extract of the suspected stain, to which has been added 0·1 c.c. of physiological salt solution; (5) the extract, with normal rabbit serum 0·1 c.c. added to it; and (6) 2 c.c. of salt solution, with 0·1 c.c. of antiserum. In the tube containing the human blood-solution, and in the tube containing the stain-extract, if it be of human blood, we shall have at the point of contact of the antiserum (which, as it is heavier, has sunk to the bottom) and the fluid, at latest within one or two minutes, a steamy turbidity, which is best viewed by transmitted natural or artificial light against a black background, such as may be made with a sheet of carbon-paper. Within fifteen minutes this steamy turbidity will have become very marked, so that even the untrained eye can detect it, and it will be seen to extend up into the solution.

As noted by Uhlenhuth and Beumer, *the entire reaction should take place at room temperature, and must be at an end within twenty minutes,* for forensic work. The reactions which take place after half an hour are very interesting to the biologist, but of no use to the medico-legist, who is concerned with the specificity of the test, and not with the 'mammalian reaction,' as Nuttall calls it, which shows the relationship which exists between various species.

All the tubes must be viewed in a rack together, and all the control-tubes must show perfectly clear contents.

Uhlenhuth and Beumer stated that, in contrast to the results obtained by Kister and Wolff, Strube, and Kratter,

they had never been able to obtain the reaction with a heterologous blood when the test was carried out in this manner. If a high-potency anti-human serum in large amount be added to a concentrated solution of a heterologous blood, a precipitate is formed; but this is smaller in amount and slower in appearance than that produced in a similarly concentrated solution of human blood, and therefore a mistake cannot arise. Kratter subsequently withdrew all objections to the test.

Hauser used corpse serum, as Ziemke had done, and also placental serum for the immunization of rabbits. In each test that he performed he used—

1. A tube containing the stain-extract alone (physiological salt solution).

2. A tube containing the stain-extract *plus* antiserum.

3. A tube containing a solution of human blood alone.

4. A tube containing a solution of human blood, *plus* antiserum.

5, 6, 7, etc. Tubes containing solutions of other bloods *plus* antiserum.

He found that the extracts made with salt solution were better than those made with the 0·1 per cent. soda solution used by Ziemke.

For filtering the extract he held the filter-paper, previously wetted with salt solution, directly over the test-tube, using no glass or other filter. All the test-tubes were filled up to the same level with salt solution, and then 1 part of antiserum to 30 parts of the extract or blood-solution was pipetted into the tube.

For forensic cases, in which the material for examination may be very small, he recommended the use of capillary-tubes for the test. Only new tubes must be used, and these must be cleared by having a little distilled water boiled in them, the explosions caused by the bursting of the bubbles when the tubes are heated in the flame causing the removal of any particles which may lie in the lumen of the tubes. The tubes should be examined with a magnifying-glass after this to ensure their being thoroughly clean. They are then

filled by capillary attraction to a convenient height—2 to 5 cm.—with the various solutions, the end that has been wetted being carefully dried with tissue-paper, and they are then laid on a glass slab, with the filled ends projecting over its edge. By means of a sterilized capillary-tube a drop of the antiserum is then brought on to a carefully polished and sterilized slide, which is inclined so that the drop runs to its edge, which is then brought into contact with the end of one of the capillary-tubes on the slab. This tube should be nearly horizontal, and the slide should be well tilted, so that the drop may be sucked up into the tube without the intervention of an air-bubble between it and the solution in the tube. If the tube be quite horizontal too much antiserum will flow into it, and if it be too upright some of the solution will escape on to the slide.

The end of the tube is then carefully dried with tissue-paper and sealed with plastiline—a modelling clay which does not easily dry. For each capillary-tube a fresh slide and fresh drop of antiserum are required.

Soon, even at room temperature, there is seen in the human blood-solution tube a ring of turbidity at the point of contact of antiserum and solution, which becomes gradually more marked, extending up into the solution. Finally a precipitate is formed, the antiserum and more distant part of the solution remaining clear. The magnifying-glass must be used to examine the tubes, to avoid a mere cloudiness of their outside or specks of dirt being taken to be a precipitate.

Hauser stated that he believed that when the test is carried out in this way it does not matter whether the proportion of antiserum to solution be 1 : 30 or 1 : 3; for in testing solutions of the blood of the calf, pig, sheep, goat, dog, fowl, goose, and pigeon, he never got a precipitate within twenty-four hours, the solutions remaining quite clear when he used 1 part of anti-human serum to 3 of solution. Uhlenhuth speaks in high terms of this method of carrying out the test. Biondi used tubes of 10 cm. length and 5 mm. bore, into which he put 1 to 2 c.c. of the solutions, and to this quantity added 1, 2, or 3 drops of antiserum.

A. Robin recommends that the test be carried out with hanging-drop preparations, as the reaction is sooner appreciable by means of these. He found that an antiserum, which when fresh caused the formation of a visible test-tube precipitate within thirty minutes, after it had been kept with chloroform for four months required two hours for the production of such a precipitate in a test-tube, while within fifteen minutes the reaction was evident in a hanging-drop preparation.

The hanging-drop method has also been used by Grünbaum, Tchistovitch, Tarchetti, Modica, and Biondi, but I consider that its employment offers no advantage over the use of test-tubes.

We now may discuss the means of obtaining antiserum. The animals used may be *rabbits*, which stand the process well; *guinea-pigs*, which yield but a small quantity of serum; *dogs* (Arthus and Vansteenberghe), which take a long time to immunize and require a large amount of material, on account of their size; or *fowls* (Ewing), which are easy to procure, and yield a fair amount of serum; or *horses*, as de Lisle appears to have used (I have not read the original paper by him). Working with guinea-pigs for other purposes, Klebs swaddled them in cloths, leaving only the belly—the site of injection—exposed. Voges is reported (I cannot trace the reference) to have devised a tin case into which the guinea-pig might be slipped head first, so that the fore part of its body was fixed. Friedberger had a left breast-pocket sewn on the outside of his laboratory coat, and thrust the guinea-pig into this head first, its back being towards his right. He then steadied the animal's hind-quarters with the middle and ring fingers of the left hand, and the site of injection in the belly with the thumb and index finger.

For the immunization of fowls, all that I have found to be necessary is to have the bird held breast up by an assistant, who grasps the legs with one hand and keeps the wings well in to the sides with the other. The skin over the abdomen is snipped with scissors, and through the small wound thus made the blunted needle of a Hübner's syringe is thrust with a boring

movement into the abdominal cavity, into which the serum then drains from the barrel of the syringe, but little compression of the indiarubber ball being required, 3 to 6 c.c. being the dose. For the immunization of rabbits the methods of injection are these:

Subcutaneous: Bordet, Dieudonné, Dubois, Hauser, Loele, Mertens, Okamoto, Stern, Stoenesco, Uhlenhuth, Wassermann, and others.

Intravenous: Camus and Gley, Kister and Wolff, Leclainche and Vallée, Mertens, Graham-Smith and Sanger, Strube, Tchistovitch, and others.

Intraperitoneal: Bordet, Cantacuzène, Ewing and Strauss, Hauser, Myers, Nuttall, Remy, Graham-Smith and Sanger, Tchistovitch, Uhlenhuth, and others.

For *subcutaneous* injections any convenient part of the animal may be taken—in the rabbit the back near the rump and the loose skin of the flank are good sites—and after preliminary disinfection of the place the injection is made with a Hübner's syringe. For the rabbit 5 to 8 c.c. will be enough for a dose, repeated at intervals of five to seven days. Loele has recently revived the subcutaneous method, which had fallen into disfavour owing to the liability to the formation of abscesses, whose cause Pfeiffer found to be the tissue necrosis caused by the hæmolysins present in the heterologous serum injected.

For *intravenous* injections the marginal vein of the rabbit's ear is chosen. The fur over this is clipped, and after the place has been disinfected, the skin is snipped over the vein with sterilized scissors, and the needle introduced into the vein. This manœuvre is greatly facilitated by using ether as the disinfectant, for the vein then tends to swell up and become prominent. Great care must, of course, be taken to avoid injecting air into the vein, and the rabbit must be held perfectly still during the operation, else the needle may pierce the other side of the vein, or even pass right through the ear. Small rabbits are of no use for intravenous injections, as the operation is rendered difficult by reason of the smallness of their ear-veins. After the needle is withdrawn, the site of

the injection is nipped with the finger-nail to cause hæmostasis. The great advantage of intravenous injections is the small quantity of serum required, and the rapidity of the production of an antiserum. The dose of human serum given intravenously should not exceed 1·5 to 2 c.c. per kilo of the rabbit's weight.

Intraperitoneal injections are easy to give, but of course more serum must be injected than is needed for intravenous injection. Nuttall recommends that an area 5 cm. square be shaved on the left side of the rabbit's belly, after the fur has been thoroughly lathered with lysol soap. The skin is then punctured with a scalpel, and through the small wound the blunted needle of the syringe is slowly bored through the abdominal parietes, and the injection is made. After withdrawal of the needle the wound is sealed with a drop of compound benzoin tincture.

To avoid wounding the intestines, Stevenson and Bruce used a bent needle with the eye on the convexity, and thrust this into a pinched-up fold of the abdominal wall; Sobernheim pinched up a fold on the belly-wall, thrust a straight needle into this, withdrawing the needle until he felt its point free in the cavity; R. Pfeiffer snipped the skin of the belly with scissors, and bored the needle through the wound thus made, the needle having been previously blunted; and Friedberger follows this plan, but uses a syringe whose end is drawn out into a fine bevelled point, the needle and syringe being thus in one piece.

For the blunting of the needle all that is required is to touch the point all round with a dry hone. If the needle point be held more or less perpendicular to the hone and scratched along it, the point will be so blunted that the abdominal skin must be snipped or punctured, while a needle blunted as I have indicated will easily enough pass through the skin.

For intravenous injections the rabbit is allowed to crouch on the table with its head towards the operator. The assistant then covers its eyes, and at the same time steadies its head with his hand. With the other hand he may, if neces-

sary, steady its body; but, as a rule, the rabbit does not appear to feel the prick of the needle, and certainly it does not struggle as if in 'pain,' whatever Dr. Kenealy and her like may say.

For intra-abdominal injections the assistant holds the rabbit stretched out head downwards, so that the intestines may lie out of the way of the needle, which is slowly bored through the abdominal wall. As a rule, the fluid flows of itself into the cavity, when the cock of the fitting of the india-rubber ball of the Hübner's syringe is turned on. I have found that disinfection of the puncture site with ether is sufficient, without afterwards sealing the puncture. From 6 to 10 c.c. of serum may be injected in this manner at a time. Care should be taken not to heat the metal fittings of the ball cap too much, else (as has happened to me) the metal may adhere tightly to the glass barrel of the syringe, and defy all subsequent attempts at removal.

The syringes and needles having been washed out with sterile salt solution after use, are then washed out with absolute alcohol, and kept in a glass jar containing absolute alcohol. Of course the ball caps must not be thus treated.

As, especially when intravenous injections are made, serious symptoms may arise after the injection—the animal may die immediately, or within a few hours—it is of importance to keep within what is considered to be the minimum lethal dose of a serum per kilo of the animal's weight. Uhlenhuth has been at some pains to work out this, and from his article on the subject* I have compiled the table on p. 124.

I am inclined to take the figures given by Uhlenhuth as the extreme limit of safety. What we desire to do is to keep our animals in good health. A good criterion of their state of health is their weight, which should be noted before the injection and at intervals thereafter, the injections being intermitted when a pronounced loss of weight is observed, it being merely a waste of good material to use serum for injecting a sick animal.

* He does not give any references to the works of the authorities cited by him.

Kind of Serum, and its Minimum Lethal Dose per Kilo for a Rabbit.					Authority.
Human.	Horse.	Sheep.	Ox.	Pig.	
C.c.	C.c.	C.c.	C c.	C.c.	
10	—	12	8	—	Rummo and Bordoni
8-9	—	—	—	—	Ludwig and Savor
10	—	—	—	—	Chamberlend and Tarnier
$9\frac{1}{2}$-11	—	—	—	—	Albu
$12\frac{1}{2}$-18	—	—	—	—	Mairet and Bosc
17	324	—	9·22	—	Guinard and Dumarest
23	—	—	—	—	Leclainche and Remond
27	—	—	—	—	Charrin
—	44	—	8	35	Weiss
7-10	—	11	6	12	Uhlenhuth

The fluids which may be used for immunization for obtaining a precipitating serum are these :

Fluid.	Used by, amongst others.
1. Defibrinated blood ...	Ewing and Strauss, Nuttall, Uhlenhuth.
2. Cell-free serum ...	Dieudonné, Nolf, Stern, Wassermann and Schütze.
3. Albuminous urine ...	Dieudonné, Leclainche and Vallée.
4. Ascitic exudation ...	Arthus and Vansteenberghe, Corin, Nedrigailov, Schütze.
5. Hydrocele fluid ...	Schütze.
6. Pleural exudation ...	Butza, Dieudonné, Kamen.
7. Corpse blood	Hauser, Modica, Nuttall, Schattenfroh, Weichardt, Ziemke. (Carrara condemns this, as all his animals injected with it died. I have not had such untoward results.)
8. Corpse blood and formalin - salt solution, equal parts ...	Loele.
9. Placental serum ...	Hauser, Nedrigailov, Okamoto, Robin.
10. Pericardial fluid from corpse.	

No. 10 I have used for intravenous, as well as intraperitoneal injection, the fluid being obtained by puncturing the pericardium and abstracting the fluid with a sterile syringe; but the amount of antigen in corpse fluids is very small. Fluids from the living are best, and, on the whole, *placental serum* is the most satisfactory fluid, as it can be obtained in fair quantities, and may be relied upon to be sterile if collected thus:

After the child is born and the umbilical cord is cut, the placenta is drained of blood while it is still in the uterus by the cord, previously disinfected, being cut, and the blood allowed to run into a sterilized Erlenmeyer flask in which are some sterilized (by being made red-hot) steel-shavings. The flask, after the blood has ceased to flow, is stoppered with a plug of cotton-wool in the usual way—the mouth of the flask and the wool being previously passed through the spirit or Bunsen flame—and the contents of the flask are well shaken, so that the steel-shavings may collect all the fibrin. The defibrinated blood is then decanted into sterilized test-tubes —from 35 to 45 c.c. or more will be present—and the tubes are left standing in the ice-chest for twenty-four hours, the serum being then pipetted off into smaller tubes, or bottles—duly sterilized, of course—and stored for use.

Or the placental blood may be allowed to clot in the Erlenmeyer flask—in which there need then be no steel-shavings—the flask being gently tilted as it lies in the ice-chest, the serum being pipetted off next day. By either of these methods a perfectly clear sterile human serum may be obtained.

Larger animals whose blood is required for injection may be bled by thrusting a sterilized trocar into the jugular vein, which has been rendered prominent by pressure. Smaller animals may be bled from the femoral or carotid at once, two or more being thus bled if their serum be required in fair quantity.

The animals which are immunized should be marked. Various expedients have been suggested, even elaborate diagrams of the animals' markings being made; but the best and simplest method appears to be that employed by

Nuttall, who tattoos the animal on the inside of the ear with a distinctive mark. A register should be kept in which are noted: (1) the kind of animal; (2) its mark; (3) the serum injected; (4) the quantity; and (5) the method of injection—the dates of the various injections, and the potency and quantity of the antiserum obtained, with the weight of the animal before and during treatment, being also noted.

The antiserum obtained may be at once frozen after it has been pipetted into phials of 10 c.c. capacity. It will then keep good for an indefinite period. For this freezing a refrigerator in which the temperature is always below 5° below zero C. is required. The question of the preservation of antisera by other methods for the tropics at least is not yet settled, as we shall presently see.

As to the number of injections required, opinions vary. Weichardt reported that he obtained an excellent antiserum after five daily injections of 1 c.c. of blood, mixed with 0·5 per cent. of its volume of phenol, the animal being bled on the eighth day. Uhlenhuth's practice appears to be the giving of an injection every sixth to seventh day. Nuttall recommends that the injections be made every fifth to seventh day, the dose being gradually increased in the case of sera whose lethal dose is unknown.

Undoubtedly excellent results are obtainable by means of intravenous injections of 1·5 to 2 c.c. per kilo, repeated every third day, the animal being bled for testing the immune power of its serum on the sixth day after the third injection. And good results are also obtained by intraperitoneal injection of 2 to 5 c.c. into fowls, the injections being repeated every fifth day, and the bird bled from the wing-vein on the sixth day after the third injection, for testing the immune power of its serum. But the individuality of the rabbit plays a great part in the manufacture of the antiserum: some rabbits are easily immunized, while others are very refractory and useless for forensic purposes.

Loele states that where he failed to secure an immune serum by intraperitoneal injections, he secured it by subcutaneous injections of blood and formalin. I have not tried

this method of treatment, which, however, may be of use when other means have failed.

The testing of the immune power of the animals' serum is carried out by means of known dilutions of the homologous serum.* Should the precipitating power be anything above what acts on 1 : 1000 dilution of homologous serum, the animal should be fully bled, as it is not likely to improve by receiving more injections. And it is a fact that too large doses in the beginning do not hasten the production of the immune body, but rather the reverse. This point it is well to bear in mind.

Although, as we shall see, some authorities recommend that when an animal is found to yield a good antiserum it should be kept, but a little blood being taken from it from time to time, the fact that Tchistovitch, working with eel serum, and Nuttall, working with ox and sheep serum, found that long-continued treatment of rabbits leads to the disappearance of precipitins from the blood, is, to my mind, sufficient to induce one to bleed one's rabbits to death as soon as they are found to give a good antiserum. As Nuttall wrote: 'There is, therefore, a point in the treatment of animals when, for purposes of obtaining an antiserum, a maximum of reaction is reached, and the animal should be bled. This can be determined by periodic bleedings from the ear-vein.'

As to the use of rabbits alone, Nuttall has much that is cogent to say; but I am not sure that the domestic fowl, or other birds, may not later be found to be at least fairly satisfactory as a source of antiserum—for the reason that the birds are so far removed from man and the other animals whose blood will usually have to be looked for. So far as I am aware, the work of Ewing in this direction has not been followed by any other observers, save Uhlenhuth, who used fowls for special reasons, and myself.

For obtaining the antiserum various procedures have been adopted.

* The blood is collected in a U-shaped capillary-tube, whose contents are then centrifugated, the cell-elements being thus collected at the bend of the tube. The supernatant serum is then removed and tested.

Stoenesco anæsthetized his rabbits with ether, opened the thorax and pericardium, and allowed the blood to drain from the heart into a sterilized tube, in which it was allowed to clot, the serum being pipetted off next day. He found that the serum was slightly cloudy, until the ether had evaporated, when it became clear and fit for use.

Camus and Gley received the animal's blood in a solution of neutral oxalate of potassium, to prevent its coagulation, and then centrifugated the mixture for several hours. They then, after decanting the superfluid, washed the erythrocytes with 1 per cent. salt solution, and took the upper part of the wash-fluid for their experiments.

Nuttall shaved the neck of the rabbit and disinfected the skin with lysol, the fur on the head and front of the chest being moistened with lysol to prevent its flying about. He then had the animal's head held well back, and the skin of the throat made tense. The throat was then cut with a sterilized knife, and the blood allowed to flow into a sterilized dish, where it was allowed to clot. After the clot had formed the dish was slightly tilted to permit of the separation of the serum, which was then pipetted off into sterilized test-tubes, from which it was pipetted into small sterile bulbs.

Graham-Smith and Sanger followed this plan, the serum being sucked up into tubes 10 cm. long and 0·5 cm. in bore, whose ends had been drawn out into fine points. The ends of the tubes were then sealed, and the tubes stored upright. When required, a few drops of serum were taken from the tubes, which were again sealed. They noted that antisera kept thus in the light in a room were as good as those kept in the ice-chest in the dark.

Uhlenhuth has recommended the abstraction of a little blood from the rabbit by means of the artificial leech when it is required, the rabbit being kept alive.

A. Robin recommended that the rabbit be bled from the femoral artery, and that, after a sufficient quantity of blood has been obtained, the wound should be sewn up.

Vincent recommended that when a rabbit is found to yield a good antiserum it should be bled only a little from time to

time, and should receive a fresh injection of serum every eighth day to keep up the potency of its serum.

I have found the following method of obtaining an antiserum to be satisfactory—it is the method employed in Frankfort: The hind-leg of the animal on which it is desired to operate is fixed by being bound to some article of furniture, to steady the site of operation. The animal is held on the stretch, head upwards, by the assistant, and with a touch of the knife on the—it may be—previously washed inguino-femoral region, the skin is easily removed, exposing the artery, which is opened with a sterilized knife, the blood being collected in an Erlenmeyer flask, in which it is allowed to clot. The flask is then placed in the ice-chest, being tilted, and left overnight, the serum being pipetted off next morning into sterilized phials of small capacity, which are sealed, the corks (which have been sterilized) being carefully charred in the flame, and then sealed with paraffin, which preserves the inscription on the cork. The animal should have been made to fast from the evening of the day previous to the bleeding. It was Uhlenhuth who first noticed that there is a connexion between the processes of digestion and opalescence of serum, and as what we require is as clear a serum as possible, we cause the animal to fast. A hæmoglobin-tinted serum is, as Loele notes, likely to be a clear one; but Hauser believes that the presence of even a trace of hæmoglobin affects the power of an antiserum adversely.

The question now arises as to how we may best preserve the antiserum which we have obtained.

1. **Freezing** has yielded excellent results in the hands of Nuttall, Sachs, and others, the sera so preserved remaining potent for years.

2. **Drying** has been recommended by Modica, who dried his sera *in vacuo* or in air at 37° to 40° C., and by de Nobele, Corin, Grigorjew, Kolle and Wassermann, and others. Nuttall, however, found that he very often obtained only a cloudy solution when he dissolved the dried residue in physiological salt solution. Biondi dried his antisera over sulphuric acid or quicklime. Nuttall, W. Richardson, and Jacobsthal

preserved antisera by saturating filter-paper with the serum and allowing this to dry. Jacobsthal used paper No. 571, manufactured by Schleicher and Schüll, and kept his antiserum-paper in the dark. Black filter-paper is used by v. Eisler—on each slip 0·1 c.c. of antiserum—the drying being effected by keeping the slips in the thermostat at 36° C. for two to four hours. He reports that if the papers be protected from light and damp, no loss of power takes place after three months. When the test is carried out the strip of paper is put into the serum solution or stain-extract, and the tube shaken for a minute to dissolve the antiserum. I find that no shaking is required, but that without it the reaction takes a longer time. The object of using black paper is to show up by its means any turbidity which may be produced.

Chemical Precipitation.—Corin saturated his antiserum with magnesium sulphate, to precipitate the paraglobulin, which he then redissolved, and again precipitated from its solution, the precipitate then obtained being dried and stored for use, to be dissolved in physiological salt solution when required. He stated that if, instead of magnesium sulphate, ammonium sulphate be used, albumin is thrown down with the paraglobulin, and that this is a drawback, as the paraglobulin in this combination is not easy of solution in the small quantities of salt solution, which are employed, in order to obtain as concentrated a solution of the antiserum as possible.

Chemical Preservatives.—Chloroform has been recommended by Biondi, Loele, and others, the quantities used being 1 to 5 per cent. of the volume of antiserum; but Corin and Nuttall do not approve of its use, as they found that it tended to make the antiserum cloudy.

Phenol, added in quantities of 5 per 1,000 volumes of antiserum, has been praised by many, among whom Uhlenhuth is chief.

Trikresol was tried by Nuttall, who soon gave up its use. Fluoride of sodium was added in the proportion of 3 per cent. to their antisera by Arthus and Vansteenberghe,

who found that antisera thus treated kept good for three months.

Nuttall, whose experience has been vastly greater than that of others, does not approve of the use of preservatives, and nowadays the tendency in most quarters is to follow his lead, and to rely on strict asepsis for the preservation of antisera. Perrando has tried—(1) drying antisera, (2) precipitating the globulins by Corin's method, and (3) storage in sterile flame-sealed tubes, and finds that the last mode of preservation, first carried out by Nuttall, is by far the best, as antisera thus preserved keep good for seven to ten months, even when exposed to light. His results thus confirm the observations of Graham-Smith and Sanger.

I have carried out experiments with antisera stored in sterile flame-sealed tubes by Nuttall, and with anti-human serum dried on black paper by v. Eisler. For these antisera the tests were devised with known dilutions of the homologous sera or salt solution extracts of homologous blood-stains, heterologous controls being, of course, employed in each case.

The results obtained by me were briefly as follows:

Nuttall's Antisera.—The following, kindly supplied by him, were tested: (1) Anti-orang-outang of 14.11.02; (2) anti-cat of 26.5.02; (3) anti-dog of 25.3.02; (4) anti-fowl of 7.12.04; (5) anti-ox of 5.2.02; and (6) anti-pig of 30.3.06.

The anti-cat serum tube had, unfortunately, one of its ends broken, and this was found to be sealed up by the saw-dust and cotton-wool in which the tubes were packed. The other tubes were in good condition, and their contents clear, only a slight deposit being visible in the drawn-out ends, as is always the case with sera stored thus.

All save the anti-cat serum gave good reactions with their homologous solutions—in the case of the anti-orang serum human serum and monkey blood-stain solution were used—even when the dilution was high (1 : 1,000), well within twenty minutes, the controls remaining free.

V. Eisler's anti-human serum of February, 1906, was packed in a tube over sticks of caustic potash, the tube being enclosed

in a wooden cylinder. The fragments of paper were sometimes shaken up with the test solution, and sometimes the serum was allowed to dissolve off of itself, the period of reaction after the settling of the serum at the bottom of the tube being noted. The reaction was good with a 1 : 1,000 dilution of human serum after five minutes. The controls remained free.

I have also tested various antisera which had been frozen when collected, and then thawed some months afterwards and stored in capillary-tubes, without the addition of a preservative, the tubes being flame-sealed and kept at room temperature for four months. In a few tubes bacterial growth was evidently the cause of the turbidity present, and these were rejected. The others contained good clear serum, and in all cases this was found to give a good reaction with 1 : 1,000 dilution of the homologous serum, the controls remaining free.

It is evident that, in Europe at least, if an antiserum be collected aseptically and stored in sterile capillary-tubes, which are flame-sealed and kept at room temperature, it will keep good for five years. Whether the conditions in the tropics are favourable to this method of storage of antiserum remains to be seen.

In any case, antisera which are collected and immediately frozen will keep good for an indefinite period, whether they be absolutely sterile or not, and even if they be thawed when required and again frozen; but the temperature of the storage-place must be 5° below zero Centigrade.

We may now consider the value of the test as to its specificity. Kratter, and also Okamoto, were dissatisfied with the results obtained by them; but Kratter withdrew all objections at the annual meeting of the Naturalists' Association held at Breslau in 1904.

Stern's objections may be disregarded from the medico-legal point of view at least, for they are based on results obtained after four to five hours.

Stevenson, in a private communication, states that the test, which he has carried out many times, 'is not conclusive.'

On the other side, in favour of the specificity of the test, we have the opinions of the authorities cited in the text, of whom Nuttall, Wassermann and Schütze and Uhlenhuth are chief, and of these observers: Corin, v. Eisler, Ewing and Strauss, Florence, Grund, Hunter, Kamen, Kockel, Kowarski, Kraus, Martin, Minovici, Mirto, Patek and Bendett, Perrando, Pugnat, Robertson, da Silva and Aguiar, Strauch, and Wood. Also, as appears from private communications made to me, Knauff, Popp, Strassmann, Thoinot, and Willcox, are strongly in favour of the test. 'Ubique et ab omnibus' the test may be said to be held to be of value as a specific test. As Sachs has well pointed out, it and the complement-deviation test are absolutely impeccable in their negative phases, and the precipitin test we may safely consider to be a conclusive test of the absence of albumin of any given species in a stain, and thus a valuable check on the allegations of the defence as to the innocent way in which the suspected stains have been caused. Perhaps some day we shall have a specific test for blood-elements as a whole, however old and altered these may be in a blood-stain. Until that day dawns the Courts may safely rely on the precipitin test, whose value has been proved, and in the future, when sufficient evidence has been collected as to its efficacy, on the complement-deviation test.

But, as Hauser wrote, 'The responsibility is very great when one makes a forensic blood examination by the serodiagnostic method, and can only be undertaken by those who are thoroughly conversant with the method, and have at their command all the conditions necessary for trustworthy work.' And, as Uhlenhuth has written, 'It is, of course, necessary that all sera which are to be used for forensic blood examination should have been previously tested as to their potency, and this, in my opinion, should be done under State control. . . . For an exact blood examination we must have a serum with the State's guarantee of its fitness for use, and an experienced operator.'

That serum tests are now absolutely necessary no one who has read thus far will doubt, I believe; nor will anyone doubt

that such a powerful weapon in the hands of Justice should be entrusted only to a trained observer, who should have at his command the resources of a well-equipped laboratory and all the animals that he requires for the preparation of the various antisera needed for forensic work, for on his conclusions a man's life may depend. What I have written as to technique, then, will serve only if special training and long practice are combined in the observer.

For the curious I would mention that *mummy material* has been tested by the precipitin test by Uhlenhuth and by Friedenthal, who failed—the latter with a mummy only 500 years old—to obtain the reaction for human albumin. On the other hand, v. Hansemann obtained the reaction with mummy material that was 4,000 years old, and J. Meyer (*Münchener med. Woch.*, 1904, p. 663) with mummy material 5,000 years old, while Friedenthal established the relationship which exists between the mammoth and the Indian elephant of to-day.

We shall now pass to the consideration of forensic cases in which the precipitin test has been of service. Many of these cases I have culled from the literature on the subject; the others have been placed at my disposal by the authorities whose names are given against them, and to whom I am deeply indebted for their courtesy.

Cases in which the Precipitin Test was of Use in Forensic Practice.

26. Uhlenhuth.—A sheet of music was found lying near a pool of blood on the highway. On the sheet were blood-stains, which it was thought might be due to human blood. They were found to be due to pig's blood.—*Deutsche med. Woch.*, 1901, p. 499.

27. Uhlenhuth.—In the house of a man who was accused of poaching there was found a walking-stick on which were stains, which he said were due to the blood of some geese, which his mother had killed and hung up, having dropped on to it. The man was suspected of having used the stick to carry home the carcasses of a roebuck and another animal

—either a fox or a hare—that he had shot. On the precipitin test being applied the stains were found to be due to hare's blood, and not to goose blood, as alleged. In this case the microscope was also of service, of course.—*Loc. cit.*

28. UHLENHUTH.—A man's corpse was found floating in the Moselle. In front of the house in which the man had lived were found blood-stains, which the precipitin test showed were due to human blood. At the trial of the man's relatives it became known that he had committed suicide, as he had often threatened to do, being worn out by the pain of the psoriasis from which he suffered, and of a cancer of the duodenum, with metastatic deposits in the liver, which the autopsy revealed. His family, in order to escape the obloquy of his having committed suicide, had removed the body from the room in which it was found and thrown it into the river. —'Das Biologische Verfahren zur Erkennung und Unterscheidung von Menschen- und Thierblut.' *Jena*, 1905, p. 39.

29. UHLENHUTH.—A man was accused of having stolen some fowls. He alleged that some blood-stains on his clothes had been caused by rabbit's blood. Microscopical examination showed that the stains were due to the blood of a bird, and when their extract was treated with anti-fowl rabbit serum, a distinct precipitate was at once obtained, while this antiserum produced only a slight turbidity after some time in solutions of the bloods of birds other than the domestic fowl.—*Loc. cit.*, p. 40.

30. UHLENHUTH.—A man was tried at Treves on a charge of murder. On his shirt, trousers, and stockings were found blood-stains, which, he alleged, were due to his having been present in the cow-house at the time that one of the cows had one of her horns wrenched off. This allegation was supported by the evidence of the man's mother; but the precipitin test revealed the fact that the stains were due, not to bovine, but to human blood.—*Loc. cit.*, p. 40.

31. UHLENHUTH.—A man was accused of having shot and robbed a waggoner. He alleged that the stains on his clothes were due to the drippings from some meat that he had bought at Saarbrücken; but the precipitin test gave a positive result

for human blood alone when an extract of the stains was examined.—*Loc. cit.*, p. 124.

32. UHLENHUTH.—In another case that was tried at Treves the following articles were found to be stained, and I give in tabular form the allegations of the accused person as to the source of the stains, and the real source of these as revealed by the precipitin test:

Stains on—	Alleged Cause.	True Cause.
Coat	Red paint	Human blood
Towel	Red sandstone	Not blood
Various articles of clothing	Blood from owner's nose	Human blood
Three knives	Meat	Not blood

—*Loc. cit.*, p. 127.

33. UHLENHUTH.—A woman was accused of having cut the umbilical cord of her child with scissors, and then drowned the child. She stated that the birth had taken place suddenly while she was at stool, and that the cord had been rent asunder. The stains on the scissors were, she said, due to her having used it first to cut up some plums, and then to cut off the head of a pigeon. The autopsy of the infant's body made it clear that the umbilical cord had been cut, and the stains on the scissors were found, on extraction, to give a precipitate with anti-human serum.—*Loc. cit.*, p. 144.

34. UHLENHUTH.—A man who had entered a claim for an allowance was found lying in bed with the bedclothes soaked with blood. He stated that he had had an attack of hæmorrhage during the night; but the precipitin-test showed that the blood on the bedclothes was bovine, and he confessed that he had emptied a bottleful of ox blood on to the bedclothes.—*Loc. cit.*, p. 145.

35. UHLENHUTH.—A man was accused of sheep-stealing and murder, and the following articles of clothing belonging to him, which were stained with blood, were examined, the sources of the stains being as shown:

Stains on—	Due to Human Blood.	Due to Sheep's Blood.
Coat, 12	6	6
Trousers, 9	7	3 (In one of the stains, of the size of a dollar, both bloods were present)
Vest, 4	4	0
Shirt, 1	1	0
Hat, 4	4	0

—*Loc. cit.*, p. 147.

36. VINCENT.—One of the horses of a battery was found to be wounded, and the artilleryman who was suspected of having caused the wound was found to be in possession of a blood-stained handkerchief. He stated that his nose had bled, and the precipitin test gave a positive reaction for human blood, and a negative reaction for equine blood, with an extract of the stains.—*Ann. d'hygiène*, 1904, p. 44.

37. De WILLEBOIS, Jhr. W. E. T. M. van der DOES, and HAMBURGER, H. J.—A man stabbed another at a fair. On being arrested he stated that he had acted in self-defence, and that his opponent had in the struggle been stabbed with his own knife, which he had drawn. He admitted that he owned a dagger, but this could not be found. He stated that he had thrown away his assailant's knife, but this was found in the latter's house, in a chest, and on it were found stains which the widow stated were due to her husband having used it to clean fish. On microscopical examination the stains were found to be due to mammalian blood, and the precipitin test yielded a positive result for human blood with their extract, and a negative result with solutions of stains made by the bloods of the four kinds of fish which the widow had named as having been cleaned by her husband with the knife.—*Tijdschr. voor strafrecht*, 1905, 17, p. 253.

38. SACHS.—A man was found lying in a pool of blood in a field, with twenty stab wounds in his chest, which he said had been inflicted by a butcher. The butcher, on being questioned, alleged that some stains that were found on his coat and trousers and one of his boots were due to the blood

of a cow and a pig that he had recently slaughtered. The extracts of the stains gave a positive reaction for pig's blood, and a negative reaction for human and bovine blood. The scrapings from under the man's finger-nails, which were sent for examination, were found to give a negative reaction for all three bloods.—Royal Institute for Experimental Therapeutics, Frankfort-on-the-Main. *Communicated.*

39. STRASSMANN.—In a case in which the accused person stated that some blood-stains were due to rabbit's blood the precipitin test yielded a negative result for rabbit's blood, but a positive result for human blood.—Institute for Public Health, University of Berlin. *Communicated.*

40. STRASSMANN.—In a case in which the accused person alleged that certain stains were due to pig's blood the precipitin test showed that pig's blood had not caused them.—*Ibidem. Communicated.*

41. STRASSMANN.—A man was accused of having stolen some rabbits. In his house was found a board on which were some blood-stains, which he stated were due to cat's blood. An extract of these stains was found to agglutinate rabbit's erythrocytes, and gave a positive result for cat's blood with the precipitin test. Thus it was shown that the man had told the truth about the stains.—*Ibidem. Communicated.*

42. KOCKEL.—A man stated that, as the result of an accident, he was suffering from hæmorrhage from the urinary tract. A physician in whose presence the man passed urine noticed that he poured into the vessel some blood from a phial. On being questioned he stated that this was blood which he had passed a short time previously. It was suspected to be the blood of a sheep or a pig, and the precipitin test gave a positive reaction for sheep's blood and a negative reaction for pig's blood with the blood in the phial.—Institute of Legal Medicine, University of Leipzig. *Communicated.*

43. KOCKEL.—The following articles were sent for examination:

(*a*) A gun that had been found in a wood, and which the

accused person said did not belong to him, and therefore he could say nothing regarding the stains on it. The precipitin test showed that the stains were due to human blood, and not to deer's blood.

(*b*) A pair of gloves, of which the accused person said he knew nothing. Horse's, not human nor deer's, blood had caused the stains on these.

(*c*) A knapsack, the stains on which were stated by the accused person to be due to horse's and deer's blood. This statement was borne out by the results of the precipitin test, and the reaction for human blood was negative.

(*d*) A piece of cloth, said to be stained by the menstrual blood of the wife of the accused person. The precipitin test showed the presence of human blood and the absence of horse's and deer's blood.

(*e*) An axe, the stains on which were said to be due to deer's blood. The precipitin test gave a positive reaction for deer's blood and a negative reaction for human blood.—*Ibidem. Communicated.*

44. HAMBURGER.—A man was accused of having stolen and slaughtered a goat. For examination the court at Heerenveen sent the following *pièces à conviction* in this case: a knife, on which were stains which the man stated were due to fish blood, and a knife, a piece of rope, and a pair of trousers, the stains on which, he said, had been caused by the blood of a cow. The stains on the first knife were found to be due to the blood of a fish and to the blood of a goat; the other articles were found to be stained with goat's blood. The possibility of goat's blood being present the accused person had emphatically denied. In the pockets of the trousers were found some hairs, which on examination turned out to be those of a goat.—Institute of Legal Medicine, University of Groningen. *Communicated.*

45. HAMBURGER.—A man was accused of house-breaking. On entering the house by one of the windows he had cut his hands with one of the window-panes, and had left traces of blood on the floor and on various articles of furniture, with indistinct finger-marks. The stains were shown by the

precipitin test to be due to human blood, but the finger-prints were too indistinct to permit of the conclusion that the accused person had made them.—*Ibidem. Communicated.*

46. HAMBURGER.—One of the inmates of an orphanage for girls was found to be pregnant, and stated that one of the officials was the cause of this. She also said that, in order to hide the fact of her pregnancy, he had stained her linen with blood out of a pot which was in one of the garrets, and whose contents were used by painters for preparing impressions. The stains on the linen were shown by the precipitin test to be due to a mixture of bovine and porcine blood, as were the contents of the pot. And *thus far* the girl's story was corroborated.—*Ibidem. Communicated.*

47. HAMBURGER.—A man was accused of having stabbed another, through his cap, in the head. On the cap were stains whose extract gave a positive result for human blood; but the blood on the knife was in too small amount for its source to be determined.—*Ibidem. Communicated.*

48. KNAUFF.—A knife and a handkerchief that were *pièces à conviction* in a murder case were sent for examination. The stains on the knife were found not to have been caused by blood, while those on the handkerchief were due to blood, of which an extract gave a positive reaction with anti-human serum.—Institute of Hygiene, University of Heidelberg. *Communicated.*

49. KNAUFF.—From Messkirch was sent a pair of trousers on which were suspicious stains. These were found not to be due to blood at all, so the question as to whether they had been caused by human blood did not fall to be answered.—*Ibidem. Communicated.*

50. KNAUFF.—A knapsack was suspected to have been stained with human or roebuck's blood. The extract gave a negative reaction with anti-human serum. No anti-roebuck serum was available, so the presence or absence of roebuck's blood could not be determined.—*Ibidem. Communicated.*

51. PRAUM.—Examination of stains on forty-three knives yielded a positive reaction in 60 per cent. of the cases, the failures to obtain reaction being attributed to the small

quantity of blood present, or to decomposition of the stain by atmospheric influences, and especially by rust. In twenty-seven cases of stains on clothes and two cases of stains on plants exact results were obtained in every case. — Grand Ducal Laboratory of Practical Bacteriology, Luxembourg. *Communicated.*

52. GLÜCKSMANN.—Three small fragments of a wooden box on which were stains were sent for examination as to whether the stains were due to human or chicken's blood. The precipitin test yielded a negative result for human blood, and a positive result for chicken's blood.—Institute of Hygiene and Bacteriology, University of Fribourg, Switzerland. *Communicated.*

53. MARISCAL.—In a *cause célèbre* that was tried at Badajoz several articles, on which were suspicious stains, were sent for examination. On a jacket the stains were found to give a positive reaction with anti-human serum. The stains on the other articles were found not to be due to blood.—Central Laboratory of Legal Medicine, Madrid. *Communicated.*

54. MONTALTI.—An advertisement card, on which were blood-stains, was sent for examination as to whether these were due to human or sheep's blood. The precipitin test gave a negative result for sheep's blood and a positive reaction with anti-human serum. The accused person confessed his crime.—Institute of Legal Medicine, University of Palermo. *Communicated.*

55. PERRANDO.—The most recent cases in which the precipitin test has been employed have been these:

(*a*) Blood-stains on some shirts and rags.

(*b*) Stains on linen due to dysenteric diarrhœa.

(*c*) A stain on the handle of a bludgeon which was suspected to have been used by a man who was accused of having fractured another man's skull.

In all these cases a positive reaction with anti-human serum was obtained.—Institute of Legal Medicine, University of Catania. *Communicated.*

56. FERRAI.—He was called upon to examine a stain on an old much worn and washed neckerchief. No reaction

could be obtained by the precipitin test, and it was only with the greatest difficulty that by means of microspectroscopy the spectrum of hæmochromogen was obtained, and it could be determined that the stain was really due to blood. In the following cases the precipitin test could be applied:

(*a*) Suspicious stains on a coat in a case of robbery and murder were found to give a positive reaction with anti-human serum.

(*b*) In a murder case, which occurred at Montaggio, on the clothes of some persons who were suspected to have committed the murder were found stains, which turned out to be blood-stains, and yielded a positive reaction with anti-human serum with the precipitin test.

(*c*) In a murder case which occurred at Crocefieschi a printed book and the cuff of a shirt on which were suspected stains were sent for examination. The reaction obtained by the precipitin test was positive with anti-human serum.

(*d*) In a murder case a pair of black trousers on which were stains were sent for examination. The stains were found by the precipitin test to give a positive reaction with anti-human serum.—Institute of Legal Medicine, University of Modena. *Communicated.*

57. E. Martin.—In a murder case in which five or six persons were accused, on the clothes of one of them were found stains which seemed to be due to blood, and Martin was called upon to examine these. On a pair of velvet trousers near the right-hand pocket he found a very small stain, which on examination proved to be due to blood; but the reaction obtained by the precipitin test was not a decided one, so he was unable to affirm that the stain was due to human blood.—Laboratory of Legal Medicine, University of Lyons. *Communicated.*

In this case it is probable that the small quantity of blood was the cause of the non-determination of its source.

58. Ewing.—Blood-stains on clothing, wall-paper, and earth removed from beneath the decapitated trunk of a body five months after death were sent for examination. The stains on the clothing gave a positive reaction for human

blood. Those on the wall-paper were not suited for examination, as the extract of the paper itself, owing to the chemicals which it contained, gave a precipitate with various antisera. The precipitating agent was not determined, but the extract of the paper was strongly alkaline. The stains were held to be of buccal origin, as they were mixed with mucus and flat epithelial cells. The earth-stains yielded hæmatin chloride crystals with great difficulty, but gave a negative result repeatedly with the precipitin test. This earth was a sandy loam, mixed with fragments of grass, roots, and leaves, and had been exposed to the weather for months.—Department of Pathology, Cornell University Medical College, New York. *Communicated.*

59. EWING.—Large blood-stains on a coat and some small blood-stains on a pair of rubber boots yielded a positive reaction for human blood. With anti-human chicken serum diluted 1 : 10 the stains gave a positive reaction, while blood of a *Macacus rhesus* did not. The accused person alleged that the blood on the coat was due to chicken blood, but microscopical examination showed that the erythrocytes in the stains were like those of human blood. The accused person was acquitted [! S.].—*Ibidem. Communicated.*

60. EWING.—Some Italians were arrested, and on their knives were found stains which were found to be due to blood, and by the precipitin test an extract of the stains gave a positive reaction for human blood. The case never came to trial.—*Ibidem. Communicated.*

61. The following blood-stained articles were sent for examination: a pocket-knife, a roll of paper money, an undershirt, a pair of trousers, and a cuff. The test of the stain on the cuff was interfered with by the presence of starch. The other stains gave positive reactions for human blood, and the defence admitted this origin of the stains. The accused person was convicted, and confessed his guilt.—*Ibidem. Communicated.*

In the testing of the stains on the pocket-knife a curious difficulty was encountered. Repeated tests with a strong extract of the stains in 0·8 per cent. salt solution failed to

give a reaction with anti-human serum, but precipitated the normal rabbit serum used as a control. Ewing changed all his apparatus, and the salt (Kahlbaum's) that he was using, and obtained the same results. He then went to another laboratory, and obtained the same results again. Finally, by using very diluted extracts of the suspected stains he obtained good reactions with anti-human serum, while no reaction was obtained with beef serum or normal rabbit serum. He considers that this was, perhaps, a case in which solution of the precipitate occurred in excess of precipitogen, and that the reaction with normal serum might have been due to the presence of some chemical on the knife.—*Ibidem. Communicated.*

62. STEIN.—A knife whose blade was stained on both sides was sent for examination. The stains were found to be due to blood, and their extract, made with salt solution, gave a positive reaction with anti-human serum. — Analytical Chemical Laboratory, Copenhagen. *Communicated.*

63. STEIN.—A shirt that was sent for examination was found to have blood-stains on the arms and shoulders. The extracts of the stains, made with salt solution, gave a positive reaction with anti-human serum.—*Ibidem. Communicated.*

64. BEUMER.—In a house that had been burnt down there were found some fragments of *bone*—of which the largest was only 6 cm. long—which were suspected to be human. As the fragments were so small, their origin could not be determined anatomically. They were somewhat charred, but pieces of soft tissue were still present, and these, on removal, were digested for four days in salt solution, which then foamed on being shaken. The solution was filtered through a Berkefeld filter, and the clear filtrate was tested with anti-human, anti-pig, and anti-ox sera, the reaction being positive for the last-named antiserum only.—*Zschr. f. Mediz-Beamte*, 1902, p. 829.

BIBLIOGRAPHY

The works marked with an asterisk were not accessible in the original.

BIBLIOGRAPHY

I.—Older Authorities whose Works contain no Special Reference to the Importance of Blood-stains from the Medico-legal Point of View.

Alix, M. F.: De quæstionibus medico-legalibus. Thesis. *Erfordiæ*, 1774.

Belloc, J. J.: Cours de médecine légale. *Paris*, n.d.

Bernt, J.: Systematisches Handbuch der gerichtlichen Arzneikunde. *Wien*, 1834.

Brendel, J. G.: Medicina legalis. *Hannoveræ*, 1789.

Claudern, C. E.: Praxis medico-legalis, oder XXV ausgelesene Casus. *Altenburg*, 1736.

Fidelis, F.: De relationibus medicorum. *Panormi*, 1602.

Fodéré, F. E.: Traité de médecine légale et d'hygiène publique ou de police de santé. *Paris*, 1813.

Frank, J. P.: System einer vollständigen medicinischen Polizey. *Wien*, 1790.

Goelicke, A. O.: Medicina forensis. *Francofurti ad Viadrum*, 1723.

Grundelach, J. D.: Primæ lineæ chemiæ forensis. Thesis. *Erlangæ*, 1771.

Henke, A.: Abhandlungen aus dem Gebiete der gerichtlichen Medicin. *Bamberg*, 1822-34.

Kannegiesser, G. H.: Institutiones medicinæ legalis. *Kiliæ Holsatorum*, 1777.

Mahon, P. A. O.: Médecine légale et police médicale. *Paris*, 1807.

Male, E. G.: An epitome of juridical or forensic medicine. *London*, 1816.

Petermanns, A.: Casuum medico-legalium decas I. *Leipzig*, 1708.

Ploucquet, W. G.: Commentarius medicus in processus criminales. *Argentorati*, 1787.

Poussielgue, A. L.: Propositions sur divers sujets de médecine légale, de médecine pratique, et de chirurgie. *Thèse de Paris*, 1804.

Ryan, M.: A Manual of medical jurisprudence. *London*, 1831.

Smith, J. G.: The principles of forensic medicine. *London*, 1821.

Sondermann, J. G.: Dissertatio inauguralis sistens quæstiones medico-forenses problematicas. *Jenæ*, 1797.

Teichmeyer, H. F.: Institutiones medicinæ legalis. *Jenæ*, 1723.

Valentini, M. B.: Novellæ medico-legales. *Francoforti ad Mœnum*, 1711.

Valentini, M. B.: Corpus juris medico-legale. *Ejusdem loci*, 1722.

Wildberg, C. F. L.: Handbuch der gerichtlichen Arzneywissenschaft. *Berlin*, 1812.

Wildberg, C. F. L.: Rhapsodien aus der gerichtlichen Arzneiwissenschaft. *Leipzig*, 1822.

Zacchias, P.: Quæstiones medico-legales. *Lugduni*, 1674.

Zittmann, J. F.: Medicina forensis. *Francoforti ad Mœnum*, 1706.

II.—Authorities cited in the Text.

1. Anonymous: Instructions pour servir à déterminer les éléments constituants du sang dans les taches. *Ann. d'hygiène*, 1873. 40. 191.
2. Anonymous: Nouveau procédé pour reconnaître les taches de sang. *Journ. de chimie méd.*, 1845, 485.
3. Adler, O. and R.: Ueber das Verhalten gewisser organischer Verbindungen gegenüber Blut, mit besonderer Berücksichtigung des Nachweises von Blut. *Zschr. f. physiol. Chemie*, 1904, 59.
4. Albrecht, E.: Die Hülle der roten Blutkörperchen, ihre physiologische und pathologische Bedeutung. *Sitz.-ber. der Ges. f. Morphol. und Physiol. in München*, 1903, Heft 2.
5. Arthus and Vansteenberghe: Un procédé nouveau d'obtention et de conservation d'un sérum précipitant le sérum du sang humain. *C. r. soc. de biol.*, 1902, 251.
6. Ascoli, M.: Zur Kenntnis der Präzipitinwirkung und der Eiweisskörper des Blutserums. *Münchener med. Woch.*, 1902, 1409.
7. Axtell, E. R.: The medico-legal examination of the red stains found on the clothes of Charles Ford, with a plea for the use of the *camera lucida*. *Journ. amer. med. assoc.*, 1895, 139.

8. Barruel, J. P.: Mémoire sur l'existence d'un principe propre à caractériser le sang de l'homme et celui de diverses espèces d'animaux. *Ann. d'hygiène*, 1829, 267.
9. Belfanti and Carbone: Produzione di sostanze tossiche nel siero di animali inoculati con sangue eterogeno. *Giorn. d. R. accad. di med. di Torino*, 1898. IV. Luglio.
10. Bell, C.: Blood and blood-stains in medical jurisprudence. *Med.-leg. journ. New York*, 1892. 10. 129.
11. Bethe, M.: Beitrag zur Kenntnis der Zahl- und Massverhältnisse der roten Blutkörperchen. Schwalbe's *Morphol. Arbeiten*, 1891, 207.
12. Beumer: Die Untersuchung von Menschen- und Tierknochen auf biologischem Wege. *Zschr. f. Med.-Beamte*, 1902, 829.
13. Biffi, U.: Sulla emoagglutinina del sangue umano, e sulla tecnica della agglutinazione in generale. *Ann. d' igiene sper.*, 1903. 18. 232. (Also another work cited in the text.)
14. Binda: * Diagnosi specifica del sangue. *Giorn. di med. legale*, 1901, 4.
15. Biondi: Beitrag zum Studium der biologischen Methode für die spezifische Diagnose des Blutes. *Viertelj. f. ger. Med.*, 1902, Suppl. 1.
16. Birchmore, W. H.: The identification of the blood of individuals. *New York med. journ.*, 1900, 10.
17. Bizzozero: Manuale di microscopia clinica. *Milano*, 1882.
18. Bojanowski, C.: Beobachtungen über die Blutkrystalle. *Zschr. f. wiss. Zoologie*, 1863. 12. 312.
19. Bordet, J.: Sur l'agglutination et la dissolution des globules rouges par le sérum d'animaux injectés de sang défibriné. *Ann. Inst. Pasteur*, 1898, 688; 1899, 273.
20. Bordet, J.: Le mécanisme de l'agglutination. *Ibid.*, 1899, 225.
21. Bordet, J.: Les sérums hémolytiques. *Ibid.*, 1900, 257.
22. Bordet and Gay: Sur les rélations des sensibilisatrices avec l'alexine. *Ibid.*, 1906, 467.
23. Bourquelot and Bougault: Sur quelques nouvelles réactions de l'acide cyanhydrique. *Journ. de pharm. et de chimie*, 1897. 6. 120.
24. Boutigny: Note sur un procédé propre à faire connaître si une tache rouge est formée par le sang. *Ann. d'hygiène*, 1844. 32. 217.
25. Breteau, P.: Sur la valeur de la teinture de gaïac comme réactif des agents d'oxydation. *Journ. de pharm. et de chimie*, 1898. 7. 569.

26. Briand and Chaudé: Manuel complet de médecine légale. *Paris*, 1879.
27. Brouardel and Vulpian—*apud* Vibert (307). *q.v.*
28. Brücke, E.: Ueber die gerichtsärztliche Untersuchung von Blutflecken. *Wiener med. Woch.*, 1857, 425.
29. Bryk, A.: Blutkrystalle und ihre Bedeutung bei forensen Blutuntersuchungen. *Ibid.*, 1858, 729, 777.
30. Butza, J.: Un nouveau moyen pratique pour distinguer le sang de l'homme d'avec celui des animaux. *C. r. soc. biol.*, 1902, 406.
31. Camus and Gley: De l'action destructive d'un sérum sanguin sur les globules rouges d'une autre espèce animale. *C. r. acad. sci.*, 1898, 428.
32. Cantacuzène, J.: Sur les variations quantitatives et qualitatives des globules rouges provoquées chez le lapin par les injections de sérum hémolytique. *Ann. Inst. Pasteur*, 1900, 379.
33. Capogrossi, A.: Isoagglutinine ed isolisine del siero umano. *Ann. d'igiene sper.*, 1903, 552.
34. Carrara: D'un nuovo metodo per la diagnosi specifica del sangue umano. *Arch. di psichiat.*, 1904, 332.
35. Carrara: *Sulla diagnosi specifica di sangue umano. *L'arte med.*, 1900, Nos. 33, 34.
36. Casanti: Nouvelle manière de distinguer le sang humain de celui des autres mammifères. *Journ. de chimie. méd.*, 1848, 673.
37. Casper, J. L.: Practisches Handbuch der gerichtlichen Medicin nach eigenen Erfahrungen. *Berlin*, 1857-58.
38. Casper and Liman: *The same work. Berlin*, 1876 (enlarged and modified).
39. Cauvet: Taches de sperme et taches de sang. *Ann. d'hygiène*, 1874. 44. 117.
40. Cauvet: Attentat à la pudeur. *Ibid.*, 367.
41. Cauvet: Examen d'une bournous. *Ibid.*, 1877, 47, 284.
42. Cevidalli, A.: Sul reattivo di Schoenbein nella diagnosi generica del sangue. *Arch. di psichiat.*, 1905, 26, 144.
43. Cevidalli, A.: Un nuovo e semplice metodo per ottenere preparati permanente di cristalli di emocromogeno. *Ibid.*, 316.
44. Chevallier, A.: Examen comparatif de taches faites avec du sang d'homme et avec du sang sucé par les punaises. *Journ. de chimie méd.*, 1830, 526.
45. Copeman, S. M.: The medico-legal detection of human blood. St. Thomas's hosp. rep., 1890. 95.

46. Coraini, E.: *Contributo al capitolo di medicina legale relativo alle macchie di sangue. *Giorn. di med. leg.*, 1897, 1.

47. Corin, G.: Examen médico-légal des taches de sang. *Arch. d'anthrop. crim.*, 1901. 16. 409.

48. Corin, G.: Zur praktischen Verwerthung der Sero-diagnostik des menschlichen Blutes. *Viertelj. f. ger. Med.*, 1902. 23. 61.

49. Corin, G.: La pyridine comme moyen d'extraction des taches de sang. *Ann. soc. de méd. de Belgique*, 1904, 10.

50. Cotton, S.: Action de l'eau oxygenée, applications à la médecine légale. *Lyon méd.*, 1904, 1285. (Also another work cited in text.)

51. Creite: Versuche über die Wirkung des Serumeiweisses nach Injection in das Blut. *Zschr. f. ration. Med.*, 1869, 90.

52. Däubler: Ueber die Unterscheidung menschlichen und thierischen Blutes durch Messung von Grössenunterschieden roter Blutkörperchen. *Viertelj. f. ger. Med.*, 1899. 17. 258.

53. v. Decastello and Sturli: Ueber die Isoagglutinine im Serum gesunder und kranker Menschen. *Münchener med. Woch.*, 1902, 1090.

54. van Deen, J.: Tinctura guajaci und ein Ozonträger, als Reagens auf sehr geringe Blutmengen. *Arch. f. d. holländ, Beitr. z. Nat. u. Heilk.*, 1861-64. 3. 228.

55. Deutsch, L.: Die forensische Serum-diagnose des Blutes. *C.-Bl. f. Bakt.*, 1901, 29, 661.

56. Dieudonné, A.: Immunität, Schutzimpfung, und Serumtherapie. 4te Aufl., Leipzig, 1905.

57. de Domenicis: Sulla ipersensibilità dello spettro dell' emocromogeno. *Morgagni*, 1904, 135.

58. de Domenicis: Valore e tecnica della ricerca dell' emocromogeno. *Giorn. di med. leg.*, 1904, 87.

59. de Domenicis: Sulla reazione Marx-Ehrnrooth. *Ibid.*, p. 145.

60. Donath, J.: Zur Kenntnis der agglutinierenden Fähigkeiten des menschlichen Blutes. *Wiener klin. Woch.*, 1900, 497.

61. Donders, F. C.: Onderzoekingen over de bloedligschaampjes. *Nederland. Lancet*, 1847-48. 3. 531.

62. Donogány, Z.: Die Darstellung des Hämochromogens als Blutreaktion mit besonderer Berücksichtigung des Nachweises von Blut im Harn. *Arch. f. path. Anat.*, 1897. 148. 234.

63. Dragendorff: Untersuchung von Blutflecken—in Maschka's Handbuch der gerichtlichen Medicin. *Tübingen*, 1881-82, 4 vols.
64. Draper, F. W. A text-book of legal medicine. *Philadelphia*, 1905.
65. Dubois, A.: Sur la dissociation des propriétés agglutinantes et sensibilisatrices des sérums spécifiques. *Ann. Inst. Pasteur*, 1902, 691.
66. Dvornitschenko: Einige Beobachtungen über die Untersuchung von Blut- und Samenflecken. *Viertelj. f. ger. Med.*, 1900. 20. 12.
67. Ehrlich, P.: Ueber die Bedeutung der neutrophilen Körnung. *Charité-Ann.*, 1887, 288.
68. Ehrlich, P.: Die Schutzstoffe des Blutes. *Deutsche med. Woch.*, 1901, 865, 888, 913.
69. Ehrlich and Morgenroth: Ueber Hämolysine. *Berliner klin. Woch.*, 1899, 481; 1900, 453, 681; 1901, 251, 569, 598.
70. Ehrlich and Morgenroth: Zur Theorie der Lysinwirkung. *Ibid.*, 1899, 6.
71. Ehrnrooth, E.: Ueber die praktische Bedeutung der Alexinfixation (Komplementablenkung) für die forensische Blutdifferenzierung. *Viertelj. f. ger. Med.*, 1906. 32. 276.
72. Ehrnrooth, E.: Zur Frage des Nachweises individueller Blutdifferenzen. *Ibid.*, 1904. 28. 64.
73. Eisenberg, R.: Ueber Isoagglutinine und Isolysine in menschlichen Seris. *Wiener klin. Woch.*, 1901, 1020.
74. v. Eisler, E.: Ueber die Konservierung präzipitierender Sera auf Papier. *Ibid.*, 1906, 494.
75. Emmert, K.: Lehrbuch der gerichtlichen Medizin. *Leipzig*, 1900.
76. Erpenbeck, R.: Ueber chemische und microscopische Untersuchung auf Blutspuren in Criminalfällen. *Viertelj. f. ger. Med.*, 1862. 21. 15. 250.
77. Ewell, M. D.: A micrometric study of 4,000 red blood-corpuscles in health and disease. *Med.-leg. journ., New York*, 1892-93. 10. 175.
78. Ewing, J.: The serum test. *Ibid.*, 1903, 36.
79. Ewing and Strauss: Precipitins and their medico-legal use. *Med. news*, 1903, 871.
80. Ferrai, C.: Sulla diagnosi specifica del sangue col metodo biologico in medicina legale. *Boll. R. accad. di med. di Genova*, 1901, 272.

81. Figari, F.: Antitossine ed agglutinine nel sangue di animali immunisati. *Gazz. degli ospedali*, 1903. 24. 814.
82. Filippi, A.: Principii di medicina legale. *Firenze*, 1889.
83. Florence, A.: *Les taches de sang, leur signification, leur importance en médecine légale. *Thèse de Lyon*, 1885.
84. Florence, A.: Les taches de sang au laboratoire de médecine légale de Lyon. *Arch. d'anthrop. crim.*, 1901, 255.
85. Florence, A.: Peut-on distinguer le sang d'un homme du sang d'un autre homme? *Ibid.*, 1904, 215.
86. Formad, H. F.: Comparative studies of mammalian blood, with special reference to the microscopical diagnosis of blood-stains in criminal cases. *Journ. comp. med. and surg.*, 1888. 9. 254.
87. Friedberger, E.: Zur Technik des intraperitonealen Injektionen. *C. Bl. f. Bakt.*, 1905, 39, 718.
88. Friedberger, E.: Zur forensischen Eiweissdifferenzierung auf Grund der hämolytischen Methode mittelst Komplementablenkung nebst Bemerkungen der Bedeutung des Präzipitates für dieses Phänomen. *Deutche med. Woch.*, 1906, 578.
89. Friedenthal, H.: Ueber einen experimentellen Nachweis von Blutverwandtschaft. *Arch. f. Anat. u. Physiol.* (*phys. Abteilg.*), 1900, 494.
90. Friedenthal, H.: Weitere Versuche über die Reaktion auf Blutverwandtschaft. *Arch. f. Anat. u. Entwicklgsges*, 1904, 387.
91. Frigerio, L.: Omicidia: perizia microscopico-chemica sopra alcuni indumenti erroneamente ritenuti macchie di sangue. *Gazz. med. ital. lomb.*, 1884, 323, 333.
92. Gantter, F.: Zum Nachweis von Blutflecken in gerichtlichen Fällen. *Zschr. f. anal. Chemie*, 1895, 159.
93. Garibaldi, G. A. G.: Manuale di medicina legale. *Milano*, 1882.
94. Gay, F.: La déviation de l'alexine dans l'hémolyse. *Ann. Inst. Pasteur*, 1905, 593.
95. Gay, F.: The fixture of alexines by specific serum precipitates. *C. Bl. f. Bakt.*, 1905. 39. 603.
96. Gengou, O.; Sur les sensibilisatrices des sérums actifs contre les substances albuminoides. *Ann. Inst. Pasteur*, 1902, 734.
97. Giese: Ueber die Beeinflüssung des spektroskopischen Blutnachweises durch die Gegenwart organischer Farbstoffe. *Viertelj. f. ger. Med.*, 1905. 30. 225.

98. Girard-Mangin and Henri: Agglutination des globules rouges par l'hydrate ferrique, le chlorure de sodium, et différents sérums. *C. r. acad. sci.*, 1904, 1461.
99. Glaister, J.: A text-book of medical jurisprudence, toxicology, and public health. *Edinburgh*, 1902,
100. Graham-Smith, G. S.: The biological or precipitin test for blood considered mainly from its medico-legal aspect. *Journ. Hygiene*, 1903, 354.
101. Graham-Smith and Sanger. *Same title. Ibid.*, 258.
102. Grigorescu, G.: Sur la possibilité de distinguer les hématies de l'homme des hématies des autres mammifères. *C. r. soc. biol.*, 1892, 325.
103. Grigorjew: Zur Frage der Technik bei der Untersuchung von Blut- und Samen-flecken in gerichtlich-medizinischen Fällen. *Viertelj. f. ger. Med.*, 1902. 24. 82.
104. Gruber, M.: Zur Theorie der Agglutination. *Münchener med. Woch.*, 1899, 1329.
105. Gruber, M.: Zur Theorie der Antikörper. *Ibid.*, 1901, 1827, 1880, 1924, 1965.
106. Grünbaum, A.: Agglutination of red corpuscles. *Brit. med. journ.*, 1900, i. 1089.
107. Grund, G.: Ueber organspezifische Präzipitine und ihre Bedeutung. *Deutsche Arch. f. klin. Med.*, 1906, 86, 148.
108. Halban and Landsteiner: Zur Frage der Präzipitationsvorgänge. *C. Bl. f. Bakt.*, 1902. 32. 457.
109. Hamburger, H. J.: Gerechtlijk onderzoek van bloed en andere lichaamsvochten. *Tijdschr. voor strafrecht*, 1904. 17. 82.
110. Hammerl, H.: Untersuchungen über einige den Blutnachweis störende Einflüsse. *Viertelj. f. ger. Med.*, 1892. 4. 44.
111. v. Hansemann: Reaktion von Blutpräzipitine. *Verhandl. d. physiol. Gres. Berlin*, 14. ii. 04.
112. Hauser, G.: Ueber einige Erfahrungen bei Anwendung der sero-diagnostischen Methode für gerichtliche Blutuntersuchungen. *Münchener med. Woch.*, 1904, 289.
113. Hayem: Du sang et ses altérations anatomiques. *Paris*, 1889.
114. Herapath, W. B.: On the use of the spectroscope and microspectroscope in the discovery of blood-stains and dissolved blood. *Brit. med. journ.*, 1869, i. 189, 217.
115. Hirschfeld, H.: Beitrag zur vergleichenden Morphologie der Leukocyten. *Arch. f. path. Anat.*, 1897. 149. 22.

116. Hitchens, A. P.: The medico-legal value of immune serum. *Med.-leg. journ. New York*, 1903, 7.
117. v. Hofmann, E.: Mord durch Erwürgen: Untersuchung von Blutspuren, wichtiger Befund in denselben. *Viertelj. f. ger. Med.*, 1873. 19. 89.
118. v. Hofmann, E.: Einiges über forensische Untersuchung von Blutspuren. *Ibid.*, 113.
119. v. Hofmann, E.: Lehrbuch der gerichtlichen Medizin *Wien*, 1885.
120. Hoppe(-Seyler), F.: Ueber das Verhalten des Blutfarb stoffes im Spektrum des Sonnenlichts. *Arch. f. path. Anat.*, 1862. 23. 446.
121. Huenefeld, F. L.: Die Blutproben vor Gericht und das Kohlenoxyd-Blut im Bezug auf die Asphyxie durch Kohlendunst. *Leipzig*, 1875.
122. Hunter, A.: On the chemical specificity of precipitins. *Journ. physiol.*, 1905, 326.
123. Husson, G.: Recherche du sang sur les vêtements qui ont été lavés. *C. r. acad. sci.*, 1883, 955.
124. Ilberg, F.: Das Blut des Menschen und der Thiere in forensischer Beziehung mit besonderer Berücksichtigung der neutrophilen Granulationen. Thesis. *Berlin*, 1895.
125. Ipsen, C.: Ein Beitrag zum spektralen Blutnachweis. *Viertelj. f. ger. Med.*, 1898. 15. 111.
126. Ipsen, C.: Ein weitere Beitrag zum spektralen Blutnachweis. *Ibid.*, 1900. 19. 1.
127. Jacobsthal, E.: Ueber trockne Konservierung agglutinierender und präzipitierender Sera. *Arch. f. Hygiene*, 1904. 18. 207.
128. Jacopi: *Elementi di fisiologia ed anatomia comparativa. *Milano*, 1808-9.
129. Janeček: *Die Grenzen der Beweiskraft des Hämatinspektrums und der Häminkrystalle für die Anwesenheit von Blut. *Agram*, 1880.
130. Joos, A.: Untersuchungen über den Mechanismus der Agglutination. *Zschr. f. Hygiene*, 1901, 422.
131. Joos, A.: Ueber die Bedeutung anorganischer Sälze für die Agglutination. *C. Bl. f. Bakt.*, 1901. 30. 853.
132. Kamen, L.: Ueber die biologische Methode des forensischen Blutnachweises. *Wiener med. Woch.*, 1904, 1533, 1572, 1625.
133. Katayama, K.: Ueber das forensisch wichtige Verhalten von Blutspuren zu verschiedener Temperatur. *Viertelj. f. ger. Med.*, 1888. 49. 269.

134. KEMPER, R.: Ueber die dem Rose'schen Verfahren bei Nachweisung von Blutspuren indicirte Bedeutung. *Ibid.*, 1863. 24. 72.

135. KISTER AND WOLFF: Zur Anwendbarkeit des serodiagnostischen Blutprüfungsverfahrens. *Zschr. f. Hygiene*, 1902. 41. 410.

136. KLEBS, E.: Zur Pathologie und Therapie der cholera asiatica. *Deutsche med. Woch.*, 1892, 999.

137. KOCKEL: In Schmidtmann's Handbuch der gerichtlichen Medizin. *Berlin*, 1905-6.

138. KÖLLIKER, A.: Microscopische Anatomie. *Leipzig*, 1854.

139. KORNFELD, H.: Handbuch der gerichtlichen Medicin. *Stuttgart*, 1884.

140. KOWARSKI, A.: Ueber den Nachweis von pflanzlichem Eiweiss auf biologischem Wege. *Deutsche med. Woch.*, 1901, 442.

141. KRATTER, J.: Ueber den Wert des Hämatoporphyrinspektrums für den forensischen Blutnachweis. *Viertelj. f. ger. Med.*, 1892. 4. 62.

142. KRATTER, J.: Ueber den forensischen Wert der biologischen Methode zur Unterscheidung von Tier- und Menschenblut. *Arch. f. Krim.-Anthrop.*, 1902, 199.

143. KRATTER, J.: Zur forensischen Serumdiagnostik des Blutes. *Wiener med. Woch.*, 1903, 24.

144. KRAUS: Ueber die diagnostische Verwertbarkeit der spezifischen Niederschläge. *Wiener klin. Woch.*, 1901, 693.

145. KÜSTER, W.: Ueber den gerichtlichen Nachweis von Blut. *Zschr. f. angew. Chemie*, 1902, 1317.

146. LADENDORF, A.: Ueber die Erkennung von Blut durch *oleum eucalypti*. *Berliner klin. Woch.*, 1880, 504.

147. LANDOIS, J.: Zur Lehre von der Bluttransfusion. *Leipzig*, 1875.

148. LANDSTEINER, K.: Beobachtungen über Hämagglutination. *Wiener klin. Rdschau.*, 1902, 774.

149. LECHA-MARZO, A.: Un nuovo processo per ottenere i cristalli di emocromogeno e di iodo-ematina. *Arch. di psichiat.*, 1905, 663.

150. LECLAINCHE AND VALLÉE: Note sur les anticorps albumineux. *C. r. soc. biol.*, 1901, 51.

151. LEFORT, J.: Remarques sur le procédé proposé par M. Taylor pour découvrir les taches de sang. *Ann. d'hygiène*, 1870. 34. 429.

152. Legrand du Saulle, Berryer, and Pouchet : Traité de médecine légale, de jurisprudence médicale, et de toxicologie. *Paris*, 1886.
153. Lesser, A.: Atlas der gerichtlichen Medicin. *Berlin*, 1883-4.
154. L(euret): Sur le principe aromatique du sang. *Ann. d'hygiène*, 1829, 2, 217.
155. Lewin and Rosenstein: Untersuchungen über die Häminprobe. *Arch. f. path. Anat.*, 1895. 142. 134.
156. Lewis, F. T.: The shape of mammalian red blood-corpuscles. *Journ. med. research*, 1903-4. 10. 513.
157. Liebermann, L.: Ueber die Guajakreaktion. *Arch. f. d. Ges. Physol.*, 1904. 104. 207, 227.
158. Liman: Eine Blutuntersuchung. *Arch. f. path. Anat.*, 1886, 104, 394.
159. Liman: Neue Versuche zur Erkennung von Blutflecken und zur Prüfung von van Deens Blutprobe. *Viertelj. f. ger. Med.*, 1863. 24. 193.
160. Linossier and Lemoine: Sur les substances précipitantes des albumines (précipitines) contenues dans certains sérums spécifiques. *C. r. soc. biol.*, 1902, 85.
161. Linossier and Lemoine: Sur la spécificité des sérums précipitants. *Ibid.*, 276, 369.
162. Linossier and Lemoine: Sur quelques conditions de l'action des sérums précipitants. *Ibid.*, 320.
163. De Lisle, J.: *Humanized horse serum for the identification of human blood. *Yale med. journ.*, 1904-5. 11. 424.
164. Loele, W.: Ueber die Anwendung von Formalin bei dem Uhlenhuthschen Verfahren. *Münchener med. Woch.*, 1906, 1053.
165. Luff, A. P.: Text-book of forensic medicine and toxicology. *London*, 1895.
166. Macnamara, F. N.: Memorandum of the identification of blood-stains. *Ind. med. gaz.*, 1873, 145.
167. Magnanimi, R.: Sulle macchie di sangue e sulla possibilità di differenziare il sangue umano da quello degli animali domestici. *Riv. di med. leg.*, 1898-9. 2. 33.
168. Malinin, J.: Ueber die Erkennung des menschlichen und thierischen Blutes in trockenen Flecken in gerichtlich-medicinischer Beziehung. *Arch. f. path. Anat.*, 1875. 65. 528.
169. Mandl, L.: Recherches médico-légales sur le sang. *Gaz. méd. de Paris*, 1842. 10. 560.

170. Mann, J. D.: Forensic medicine and toxicology. *London*, 1902.
171. Mariscal y Garcia, N.: Examen de manchas de sangre y reconocimiento del origen de ellas. *Siglo médico*, 1889. 36. 311.
172. Martin, E.: Isoagglutination beim Menschen, nebst Bemerkung zur Marx-Ehrnroothschen Blutdifferenzierungsmethode. *C. Bl. f. Bakt.*, 1904. 39. 704.
173. Marx, H.: Ueber den Nachweis von Blutkörperchen mittelst Chinin. *Viertelj. f. ger. Med.*, 1903. 26. 38.
174. Marx, H.: Die Bedeutung der Hämagglutinine und Hämolysine der Normalsera für den forensischen Blutnachweis. *Aerztl. Sachv.-Ztg.*, 1904, 436.
175. Marx, H.: Der forensische Blutnachweis. *Berliner klin Woch.*, 1905, 266.
176. Marx and Ehrnrooth: Eine einfache Methode zur forensische Unterscheidung von Menschen- und Säugetierblut. *Münchener med. Woch.*, 1904, 293, 696.
177. Masson: De l'origine du sang en médecine légale. *Ann. d'hygiène*, 1885, 385, 530.
178. Di Mattei: Azione degli ossidanti e dei riducenti sul sangue in rapporto alla reazione col metodo biologico. *Giorn. di med. leg.*, 1904, 252.
179. Mertens, V. E.: Ein biologischer Beweis für die Herkunft des Albumens im Nephritisharn aus dem Blute. *Deutsche med. Woch.*, 1901, 161.
180. Michaelis, L.: Untersuchungen über Eiweisspräzipitine. *Ibid.*, 1902, 733.
181. Michelsson, F.: Die Differentialdiagnose von Menschen- und Tierblut in der forensischen Praxis. *Bl. f. ger. Med.*, 1906, 9, 116, 204, 285, 381.
182. Minovici: Die Blutdifferenzierung vom gerichtlichen Standpunkte. *Ber. d. deutschen pharm. Ges.*, 1903, 274.
183. Miquel: Ueber die Ausmittelung von Blutspuren. *Deutsche Klinik*, 1860, 83.
184. Mirto, D.: *Ricerche microchemiche e spettroscopiche sulle macchie di sangue modificati per azioni di agenti, e sul valore dell' ematoporfirina nella diagnosi medico-legale del sangue, e delle ecchimosi cutanee. *Giorn. di med. leg.*, 1901, 93.
185. Mirto, D.: Sul valore del metodo biologico per la diagnosi specifica del sangue nelle varie contingenze della pratica medico-legale. *Riforma med.*, 1901, 855, 866.

186. Misuraca, G.: Sull' importanza della ricerca di emoglobina nell' esame delle macchie di sangue. *Riv. sper. di freniat.*, 1889-90, 5, ii., 36.

187. Modica: Azione emolitica dell' orine di animali trattati con sangue eterogeno ed influenza di agenti esterni ed interni sulla reazione. *Il Policlinico*, 1901 (*sez. prat.*), 1131.

188. Lo Monacho and Panichi: Sul fenomeno dell' agglutinazione nel sangue dei malarici. *Riforma med.*, 1901, 400.

189. Morache: Contribution à l'étude des taches du sang humain en comparaison de celui d'autres animaux. *Ann. d'hygiène*, 1880. 3. 322.

190. Moreschi, C.: Zur Lehre von den Antikomplementen. *Berliner klin. Woch.*, 1905, 1181; 1906.

191. Moreschi, C.: Ueber den Wert des Komplementablenkungsverfahrens in der bakteriologischen Diagnose. *Ibid.*, 1906, 1243.

192. Muir and Martin: On the deviation of complement by a serum and its antiserum, and its relation to the precipitin test. *Journ. Hygiene*, 1906, 265.

193. Myers, W.: On immunity against proteids. *Lancet*, 1900, ii. 98.

194. Nedrigailov. *Cited by Nuttall from *Vratch* through an American periodical.

195. Neisser and Sachs: Ein Verfahren zum forensischen Nachweis der Herkunft des Blutes. *Berliner klin. Woch.*, 1905, 1388; 1906, 67.

96. Neisser and Sachs: Bemerkungen zu der Arbeit von Professor Uhlenhuth über Komplementablenkung und Bluteiweissdifferenzierung. *Deutsche med. Woch.*, 1906, 1580.

197. Neisser and Wechsberg: Ueber die Wirkungsart bactericider Sera. *Münchener med. Woch.*, 1901, 697.

198. Neumann: *Die Erkennung des Blutes bei gerichtlichen Untersuchungen. *Leipzig*, 1869.

199. Nicolle, Ch.: Suite d'expériences rélatives au phénomène de l'agglutination des microbes. *Ann. Inst. Pasteur*, 1904, 209.

200. Nolf: Le mécanisme de la globulyse. *Ibid.*, 1900, 656.

201. Nuttall, G. H. F.: Blood immunity and blood relationship. *Cambridge*, 1904.

202. Obermayer and Pick: Ueber die chemischen Grundlagen der Arteigenschaften der Eiweisskörper. *Wiener klin. Woch.*, 1906, 327.

203. Ogston, F.: Lectures on medical jurisprudence. *London*, 1878.
204. Okamoto, Y.: Untersuchungen über den forensisch-praktischen Werth der serumdiagnostischen Methode zur Unterscheidung von Menschen- und Tierblut. *Viertelj. f. ger. Med.*, 1902. 24. 207.
205. Ollendorff, K.: Beitrag zur Technik des Marx-Ehrnroothschen Verfahrens zur forensischen Untersuchung von Menschen- und Thierblut. *Zschr. f. Med.-Beamte*, 1905, 449.
206. Orfila, M. J. B : Chimie médical. *Paris*, 1817.
207. Orfila, M. J. B.: Nouveau mémoire sur le sang, considéré sous le rapport médico-légal. *Journ. de chimie méd.*, 1827, 365, 413; 1828, 105.
208. Orfila, M. J. B.: Mémoire sur un nouveau moyen de reconnaître les taches de sang. *Ann. d'hygiène*, 1845. 34. 112.
209. Orfila, Barruel, and Chevallier: Taches de sang: rapport médico-légal. *Ibid.*, 1835, 14, 349.
210. Pacini, E.: Di un mezzo atto a facilitare l'esame microscopico delle macchie di sangue nelle questioni medico-forensi. *Ann. di chim. applic. alla med.*, 1872, 43.
211. Palleske: Eine neu Methode des Blutnachweises? *Viertelj. f. ger. Med.*, 1905, 29, 331.
212. Palleske: Die Riegler'sche Blutprobe und ihr Werth für die gerichtliche Medizin. *Aerztl. Sachv.-Ztg.*, 1905. 19. 387.
213. Paltauf, R.: Ueber Agglutination und Präzipitation. *Deutsche med. Woch.*, 1903, 946.
214. Patek and Bendett: *The new antiserum method of differentiating human from other blood, and its medico-legal aspect. *Amer. med.*, 1902, 374.
215. Perrando: Sulla durata degli proprietà precipitanti dei sieri specifici. *Giorn. di med. leg.*, 1903, 10, 162.
216. Persoz: See Orfila (206) and Anonymous (2).
217. Pfaff, E. R.: Ueber die Bestimmung des Alters der Blutflecke in Criminalfällen. *Viertelj. f. ger. Med.*, 1862. 21. 266.
218. Pfaff, E. R.: Anleitung zur Vornahme gerichtsärztlicher Blutuntersuchung. *Dresden*, 1863.
219. Pfeiffer, H.: Erfahrungen mit der Marx-Ehrnroothschen Methode zur forensischen Unterscheidung von Menschen- und Tierblut. *Deutsche med. Woch.*, 1904, 1098.

220. Pfeiffer, H.: Ueber die nekrotisierende Wirkung normaler Seren. *Zschr. f. Hygiene*, 1905, 51, 183.
221. Ponzio, F.: La reazione biologica per la diagnosi specifica del sangue. *Gazz. med. siciliana*, 1904, No. 23. (Reprint.)
222. Poore, G. V.: A treatise on medical jurisprudence. *London*, 1902.
223. Popp: Die Beobachtung und Deutung von Blutspuren bei Kriminalforschungen. *Zschr. f. öffentl. Chemie*, 1904, Heft 19. (Reprint.)
224. Pugnat, A.: Le sérodiagnostic du sang humain et son utilisation en médecine légale. *Arch. d'anthrop. crim.*, 1902, 709.
225. Puppe: Ueber das Prinzip der Conservierung anatomischer Präparate in den natürlichen Farben. *Viertelj. f. ger. Med.*, 1899. 17. 263.
226. Raspail: Sur les moyens, soit chimiques soit microscopiques, qu'on a tout recemment proposés pour reconnaître les taches de sang en médecine légale. *Journ. gén. de med., chir., et pharm.*, 1828. 102. 335.
227. Remy, L.: Contribution à l'étude des substances actives des sérums normaux : pluralité des alexines. *Ann. Inst. Pasteur*, 1903, 343.
228. Rezzonico, G.: Ancora sulla ripristinazione dei globuli di sangue. *Riv. sper. di freniat.*, 1889-90, 15, ii. 214.
229. Richardson, J. G.: On the value of high powers in the diagno sis of blood-stains. *Amer. journ. med. sci.*, 1874, 68, 102.
230. Richardson, J. G.: Note on the diagnosis of blood-stains. *Monthly microscop. journ.*, 1875. 13. 213.
231. Richardson, J. G.: Improved method of applying the microspectroscopic test for blood-stains. *Ibid.*, 1876. 15. 30.
232. Richardson, W.: Die Diagnose von Typhuskulturen vermittelst getrockneten Typhusserums. *C. Bl. f. Bakt.*, 1897. 21. 445.
233. Richter, M.: Ueber Häminkrystalle. *Viertelj. f. ger. Med.*, 1900. 20. 22.
234. Richter, M.: Gerichtsärztliche Diagnostik und Technik. *Wien*, 1905.
235. Rickmann, W.: Beitrag zur biologischen Eiweissdifferenzierung. *Zschr. f. Fleisch- und Milchhygiene*, 1907, No. 6. (Reprint.)
236. Riegler: Ref. in *Pharm. Cent. Halle*, 1905, 355.

237. Robertson, W. G. A.: Personal experiences in the detection of human blood by means of the precipitin-serum test of Uhlenhuth. *Scot. med. and surg. journ.*, 1906, 18, 321.

238. Robin, A.: A note on the employment of the hanging-drop method in the study of hæmoprecipitins. *Philadelphia med. journ.*, 1902. 10. 1019.

239. Robin, A.: A new biologic test for human blood, with a report of its employment in a recent murder case. *New York med. journ.*, 1904. 433. 500.

240. Robin, Ch.: Mémoire sur la comparaison médico-légale des taches de sang menstruel et des autres espèces de taches de sang. *Ann. d'hygiène*, 1858, 10, 421.

241. Robin, Ch.: In Briand and Chaudé (26) as to Bug-stains.

242. Robin and Salmon: Mémoire concernant l'examen à l'aide du microscope des taches de sang sur une blouse de coton bleu. *Ann. d'hygiène*, 1857, 8, 368.

243. Rose, H.: Ueber die sichere Erkennung von Blut und von Blutflecken bei gerichtlichen Untersuchungen. *Viertelj. f. ger. Med.*, 1853. 4. 295.

244. Rossel, O.: Beitrag zum Nachweis von Blut bei Anwesenheit anderer anorganischen und organischen Substanzen in klinischen und gerichtlichen Fällen. *Deutsche Arch. f. klin. Med.*, 1903, 505.

245. Roussin, Z.: Examen médico-légal des taches de sang. *Ann. d'hygiène*, 1865. 23. 139.

246. Ruffer and Crendiropoulo: Note on a new method of producing hæmolysins. *Brit. med. journ.*, 1903, i. 190.

247. Ruffer and Crendiropoulo: Note on hæmosozic sera. *Ibid.*, 1904, i. 658.

248. Sachs, H.: Ueber die Hämolysine des normalen Blutserums. *Münchener med. Woch.*, 1904, 304.

249. Sachs, H.: Die Hämolysine und die cytotoxischen sera. Lubarsch-Ostertag's *Ergebnisse der path. Anat.*, 1907, 515.

250. Schaer, E.: Neuere Beobachtungen über Blutnachweis mittelst der Guajakprobe. *Arch. der Pharm.*, 1898. 236. 571.

251. Schattenfroh, A.: Ueber specifische Veränderungen nach Harninjektionen. *Münchener med. Woch.*, 1901, 1239.

252. Schauenstein: Lehrbuch der gerichtlichen Medicin. *Wien*, 1875.
253. Schirokich: *Cited by Nuttall from *Vratch* through *Philadelphia med. journ.*, 1901, 811.
254. Schmidt, C.: Die Diagnostik verdächtiger Flecke in Kriminalfällen. *Mitau*, 1848.
255. Schöfer, J.: Blutspuren von zerdrückten Wanzen herrührend. *Wiener klin. Woch.*, 1893, 643.
256. Schönbein, C. F.: Ueber das Verhalten des Blutes zum Sauerstoff. *Journ. f. prakt. Chemie*, 1863. 89. 22.
257. Schulz, A.: Ueber die Verwendbarkeit der von Siefert angegebenen Modifikation der Guajak-Wasserstoffsuperoxyd-Reaktion zum Nachweis von Blutspuren. *Viertelj. f. ger. Med.*, 1901. 22. 104.
258. Schütze, A.: Ueber ein biologisches Verfahren zur Differenzierung der Eiweissstoffe verschiedener Milcharten. *Zschr. f. Hygiene*, 1901. 36. 5.
259. Schütze, A.: Weitere Beiträge zum Nachweis verschiedener Eiweissarten auf biologischem Wege. *Ibid.*, 38, 487.
260. Schütze, A.: Ueber die Unterscheidung von Menschen- und Tierblut mittelst der Wassermannschen Differenzierungsmethode. *Deutsche med. Woch.*, 1903, 62.
261. Schütze, A.: Ueber weitere Anwendungen der Präzipitine. *Ibid.*, 1904, 804.
262. Schütze, A.: Ueber den forensischen Wert des Neisser-Sachs'schen Verfahrens der Komplementablenkung. *Berliner klinische Woch.*, 1906, 1646.
263. Selmi, F.: Sulla fallacia del reattivo di van Deen per determinare le macchie di sangue. *Mem. accad. di sci. del. ist. di Bologna*, 1880, i. 295.
264. Siefert, E.: Ueber die Verwendbarkeit der Guajak-Wasserstoffsuperoxyd - Reaktion zum Nachweis von Blutspuren in forensischen Fällen. *Viertelj. f. ger. Med.*, 1898. 16. 1.
265. Da Silva and Aguiar: *O exame medico-legal das manchas de sangue e o methodo de Uhlenhuth. *Med. moderna*, 1902, 317.
266. Sonnenschein, F. L.: Ueber ein neues Reagens auf Blut und Anwendung desselben in der forensischen Chemie. *Viertelj. f. ger. Med.*, 1872. 17. 263.
267. Spezia: Sulla reazione del van Deen. *Gazz. med. lomb.*, 1904, 63, 337.

268. Stern, R.: Ueber den Nachweis menschlichen Blutes durch ein 'Antiserum.' *Deutsche med. Woch.*, 1901, 135.
269. Stevenson and Bruce: Eine neue Methode Flüssigkeiten in die Bauchhöhle der Versuchsthiere einzuspritzen. *C. Bl. f. Bakt.*, 1891, 9, 689.
270. Stoenesco: La differenciation du sang par le sérum spécifique. *Ann. d'hygiène*, 1902, 40, 211.
271. Strauch: Der serodiagnostische Nachweis von Menschenblut vor Gericht. *Aerztl. Sachverst.-Ztg.*, 1905. 11. 429.
272. Strube, G.: Beiträge zum Nachweis von Blut und Eiweiss auf biologischem Wege. *Deutsche med. Woch.*, 1902, 425.
273. Struve, H.: Beitrag zur gerichtlich-chemischen Untersuchung von verdächtigen Flecken. *Arch. f. path. Anat.*, 1880. 79. 524.
274. Struve, H.: Zur gerichtlich-chemischen Untersuchung verdächtiger Flecke auf Blut. *Zschr. f. anal. Chemie*, 1893. 32. 174.
275. Szigeti, H.: Ueber die Anwendung von Carbolsäure beim Nachweis von Blutspuren. *Viertelj. f. ger. Med.*, 1896, Suppl. 101.
276. Takayama, M.: Beitrag zur Hämatoporphyrinprobe. *Ibid.*, 1905. 29. 232.
277. Tamassia, A.: Sulla determinazione cronologica delle macchie di sangue. *Atti del R. ist. veneto di sci., lett., ed arti.*, 1883-84, 945.
278. Tamassia, A.: Valore delle granulazioni neutrofile dei globuli bianchi nella determinazione specifica del sangue. *Gazz. med. lomb.*, 1894, 12.
279. Tarchetti, C.: *Di un nuovo metodo per differenziare il sangue umano da quello di altri animali. *Cron. di clin. med.*, 1901, 7, 129.
280. Tarugi, N.: Osservazioni e studi intorno alla reazione di van Deen. *Gazz. chim. ital.*, 1903, ii. 216.
281. Taylor, A. S.: On the guaiacum process for the detection of blood in medico-legal cases: the antozone test. *Guy's hosp. rep.*, 1868, 431.
282. Taylor, A. S.: On the processes for detecting blood in medico-legal cases. *Ibid.*, 1870, 273.
283. Taylor, A. S.: On the detection of blood by guaiacum. *Ibid.*, 1874, 517.
284. Taylor, A. S.: Principles and practice of medical jurisprudence. *London*, 1904 (ed. by Smith).

285. Tchistovitch, Th.: Études sur l'immunisation contre le serum d'anguilles. *Ann. Inst. Pasteur*, 1899, 406.
286. Teichmann: Ueber die Krystallisation des organischen Bestandtheiles des Blutes. *Zschr. f. ration. Med.*, 1853. 3. 375.
287. Thomas, H.: Beitrag zur Kenntnis der Hämatoporphyrinprobe. *Viertelj. f. ger. Med.*, 1904. 37. 307.
288. Tidy, C. M.: Legal medicine. *London*, 1882.
289. Tourdes, G.: Sang (médecine légale) in *Dict. encyc. des. sci. méd., Paris*, 1878.
290. Uhlenhuth, P.: Zur Kenntnis der giftigen Eigenschaften des Blutserums. *Zschr. f. Hygiene*, 1897. 26. 384.
291. Uhlenhuth, P.: Das biologische Verfahren zur Erkennung und Unterscheidung von Menschen- und Tierblut. *Jena*, 1905.

This work contains most of the following articles:

292. Neuer Beitrag zum spezifischen Nachweis von Eiereiweiss auf biologischem Wege. *Deutsche med. Woch.*, 1900, 734.
293. Eine Methode zur Unterscheidung der verschiedenen Blutarten, im besonderen zum Differentialdiagnostischen Nachweise des Menschenblutes. *Ibid.*, 1901, 82.
294. Weitere Mitteilungen über meine Methode zum Nachweise von Menschenblut. *Ibid.*, 260, 499.
295. Bemerkungen zu dem Aufsatz von Kratter: Ueber den forensischen Wert der biologischen Methode zur Unterscheidung von Tier- und Menschenblut. *Arch. f. Krim.-Anthrop.*, 1902. 10. 210.
296. Gibt es ein biologisches Blutdifferenzierungsverfahren für Menschen- und Tierblut mittelst der Präzipitine? *Deutsche med. Woch.*, 1904, 584.
297. Ein neuer biologischer Beweis für die Blutverwandtschaft zwischen Menschen- und Affengeschlecht. *Cor.-Bl. f. d. deutsche Ges. f. Anthrop.*, 1904. 35. 114.
298. Der forensische Blutnachweis. *Wiener klin. Woch.*, 1904, 2009, 2071.
299. Ueber den Stand der forensischen Blutuntersuchung. *Med. Klin.*, 1905, 1, 539.
300. Ueber die Bestimmung der Herkunft von Mumienmaterial mit Hilfe spezifischer Sera. *Deutsche med. Woch.*, 1905, 213.

301. Ein Verfahren zur biologischen Unterscheidung von Blut verwandter Tiere. *Ibid.*, 1673.
302. Komplementablenkung und Blut-Eiweissdifferenzierung. *Ibid.*, 1906, 1244, 2072.

303. Uhlenhuth and Beumer: Praktische Anleitung zur gerichtsärztlichen Blutuntersuchung mittelst der biologischen Methode. *Zschr. f. Med.-Beamte*, 1903, 185, 229.
304. Utz: Der Nachweis von Blut auf chemischem Wege. *Oesterr. chem. Ztg.*, 1902, 558.
305. Utz: Beitrag zum forensischen Nachweis von Blut. *Chemiker-Ztg.*, 1903, 1151.
306. Vibert, Ch.: De la possibilité de distinguer le sang de l'homme de celui des mammifères. *Arch. de physiol. norm. et path.*, 1882. 9. 48.
307. Vibert, Ch.: Précis de médecine légale. *Paris*, 1886.
308. Vincent, H.: Le diagnostic médico-légal. Application de la méthode biologique. *Ann. d'hygiène*, 1904, 44.
309. Virchow, R.: Ueber die forensische Untersuchung von trockenen Blutflecken. *Arch. f. path. Anat.*, 1857. 12. 334.
310. Vitali, D.: *Sul rattivo delle macchie sanguigne del van Deen. *Orosi*, 1903. 26. 109.
311. Wachholz, L.: Untersuchungen über Häminkrystalle. *Viertelj. f. ger. Med.*, 1901. 21. 227.
312. Wassermann, A.: Ueber Agglutinine und Präzipitine. *Zschr. f. Hygiene*, 1903. 12, 267.
313. Wassermann, A.: Gibt es ein biologisches Blutdifferenzierungsverfahren für Menschen- und Tierblut mittels der Präzipitine? *Deutsche med. Woch.*, 1904, 416, 694.
314. Wassermann and Schütze: Neue Beiträge zur Kenntnis der Eiweissstoffe verschiedener Milcharten. *Ibid.* (Vereins-Beilage), 1900, 178.
315. Wassermann and Schütze: Ueber eine neue forensische Methode zur Unterscheidung von Menschen- und Tierblut. *Berliner klin. Woch.*, 1901, 187.
316. Weber, H.: Ueber den Nachweis des Blutes in dem Magen- und dem Darminhalt. *Ibid.*, 1893, 441.
317. Weichardt, W.: Der Nachweis individueller Blutdifferenzen. *Hyg. Rdschau.*, 1903. 13. 756.
318. Weichardt, W.: Zur Frage des Nachweises individueller Blutdifferenzen. *Viertelj. f. ger. Med.*, 1905. 29. 19.

319. Weidenreich, F.: Ueber die Form der Säugererythrocyten und die formbestimmenden Ursachen. *Fol. haematol.*, 1905, 95.
320. Whitney, W. F.: Notes on the production of the test-serum in rabbits. *Boston med. and surg. journ.*, 1902. 146. 429.
321. Whitney, W. F.: The serum-test in rabbits. *Med.-leg. journ. New York*, 1903, 33.
322. Whittier, F. N.:* Agglutination test for human blood. *Amer. med.*, 1902. 3. 96.
323. Wiehr, C.: Beitrag zur Erkennung von Blutflecken auf leinenen und baumwollenen Zeugen. *Arch. der Pharm.*, 1854. 78. 21.
324. De Willebois, Jonkheer W. E. T. M. van der Does: Eene mishandeling met doodelijk gevolg en een belangrijk deskundig onderzoek. *Tijdschr. voor strafrecht*, 1904. 17. 253.
325. Winterberg: Reference untraceable.
326. Witthaus and Becker: Medical jurisprudence, forensic medicine, and toxicology. *New York*, 1894-96.
327. Wood, E. S.: Medico-legal examination of blood-stains. *Boston med. and surg. journ.*, 1901. 145. 533.
328. Wood, E. S.: The serum test for blood. *Med.-leg. journ. New York*, 1903, 27.
329. Wood, E. S.: In Witthaus and Becker (322).
330. Woodward, J. G.: On the similarity between the red blood-corpuscles of man and those of certain other mammals, especially the dog—considered in connexion with the diagnosis of blood-stains in criminal cases. *Monthly microscop. journ.*, 1875. 13. 65.
331. Woodward, J. G.: The application of photography and micrometry with special reference to the micrometry of blood in criminal cases. *Ibid.*, 1876. 16. 144.
332. Zahn, F. W.: Die Anwendung des Wasserstoffdioxyds zum Nachweis von Blutflecken. *Cor.-Bl. f. schweizer Aerzte* 1871. 1. 322.
333. Zanelli, U.: Sulla possibilità di riconoscere, mediante i cristalli di emina, la presenza del sangue in tessuti di varia natura dopo i lavaggi soliti della pratica comune. *Atti del R. ist. veneto di sci., lett., ed. arti.*, 1885-86. 4. 425.
334. Ziemke, E.: Zur Unterscheidung von Menschen- und Tierblut mit Hilfe eines spezifischen Serums. *Deutsche med. Woch.*, 1901, 424, 731.

335. Ziemke, E.: Ueber den Werth des alkalischen Hämatoporphyrins für den forensischen Blutnachweis. *Viertelj. f. ger. Med.*, 1901. 22. 231.

336. Ziemke, E.: Ueber die ungleiche Resistenz des Blutfarbstoffes verschiedener Thiere gegen Alkalien und eine hierauf gegründete Methode zur Unterscheidung von Menschen- und Thierblut. *Ibid.*, 77.

THE END

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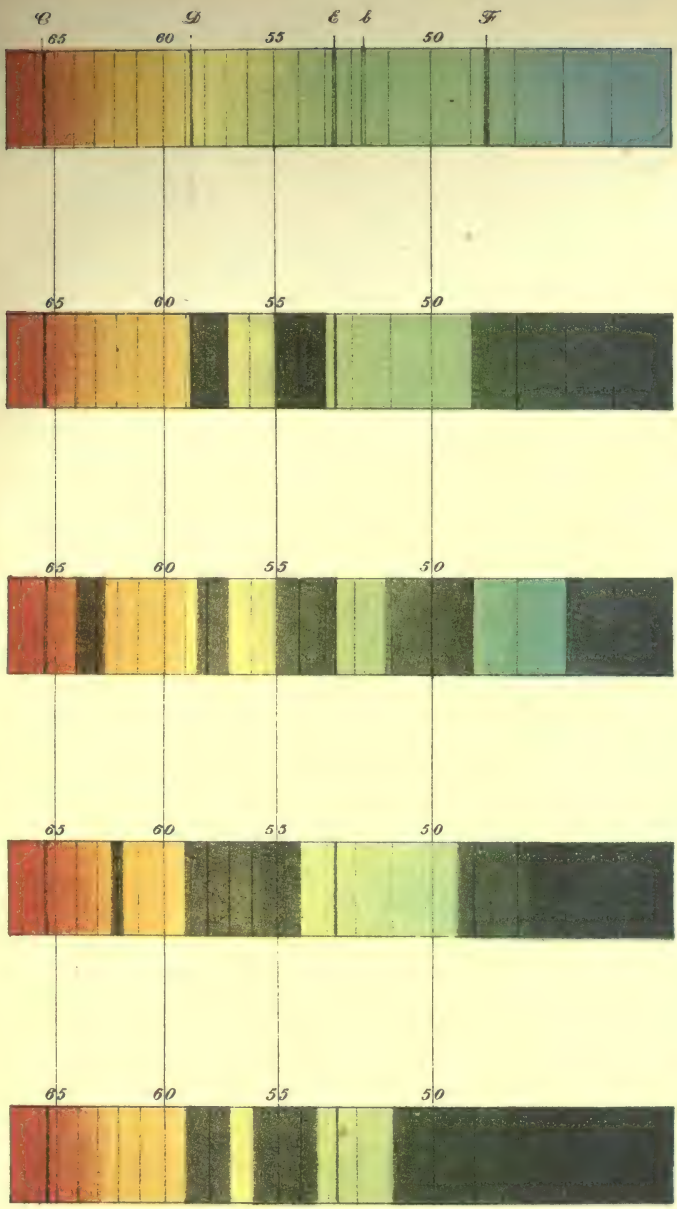
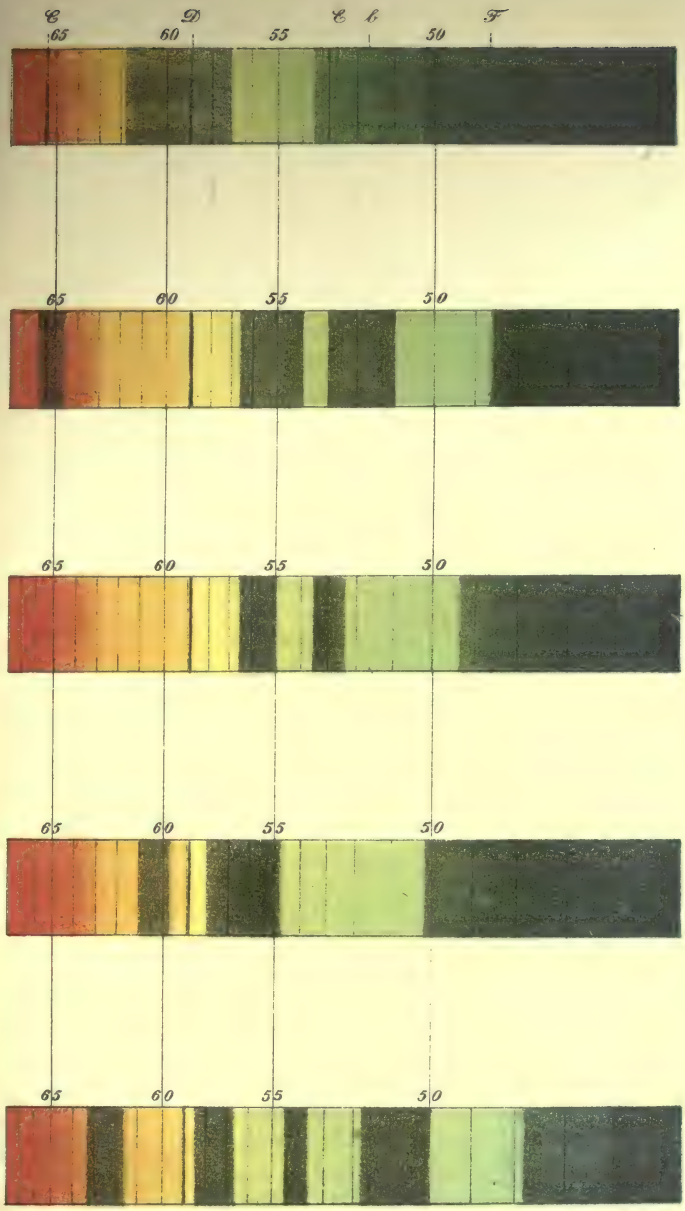
PLATE I



THE SPECTRA OF BLOOD.

Drawn on Engelmann's plates from Formánek's curves, and verified from spectra seen with a Bunsen spectroscope (the spectra from λ 45 to λ 68 alone are given).

PLATE II.



THE SPECTRA OF BLOOD.

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